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PAPERS FROM TORTUGAS LABORATORY

VOLUME XXXV

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Carnegie Institution of Washington
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Papers. v. 35

PAPERS FROM TORTUGAS LABORATORY, VOLUME XXXV

THE PHOTODYNAMIC ACTION OF DYES ON THE EGGS OF THE SEA URCHIN, LYTECHINUS VARIEGATUS

BY

DAVID HILT TENNENT, *Late Research Professor of Biology, Bryn Mawr College, and Executive Officer, Tortugas Laboratory, Carnegie Institution of Washington*

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FOREWORD

After a period of successful operation extending over thirty-five years, activities of the Tortugas Laboratory of the Carnegie Institution of Washington were terminated following the summer season of 1939. The Institution had been fortunate in securing the services of Dr. David H. Tennent as Executive Officer of the Laboratory for the three seasons following the fatal illness of Dr. William H. Longley in 1937.

Dr. Tennent started his research at Tortugas in 1909 and was a consistent contributor to the "Papers from Tortugas Laboratory." His work on hybridization of echinoderms, recorded in the early volumes, is considered a cytological classic. The present volume is devoted to what he believed to be his most significant contribution to biology. Like his early papers, it reports experiments carried out at the Tortugas Laboratory and completed by further study at Bryn Mawr College. For the last three years of his life he was able to devote almost his entire time to this research. During this period he was Research Professor of Biology, a position unique at Bryn Mawr, and one specifically created for him through the generosity of friends of the College.

In the opinion of those qualified to judge, Dr. Tennent's painstaking study, using a variety of dyes, is by far the most comprehensive that has been made of the present subject, and constitutes a fundamental contribution to the general fields of photodynamic action and the nature of the cell surface. The manuscript describing the experimental data was completed just before his death, and it is to be regretted that many of his speculations concerning the interpretation of the observations have not been recorded. Although few investigators could have distinguished the minor gradations of surface effects here so concisely reported, the significance of this paper will be evident to those with a knowledge of cellular phenomena. It is most fitting that this final volume in the Tortugas series of papers should come from one of such outstanding scientific accomplishment and from one who had been so closely identified with the work of the Laboratory.

Dr. William L. Doyle, of Bryn Mawr College, has kindly offered on behalf of his colleague to see the volume through the press and to supply such additional information as may be sought with regard to Dr. Tennent's work.

VANNEVAR BUSH, *President*
Carnegie Institution of Washington

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The Photodynamic Action of Dyes on the Egg of the Sea Urchin, Lytechinus variegatus

INTRODUCTION

The work recorded in this paper was begun as a study of the experimental modification and control of cell division in the egg of the sea urchin, *Lytechinus variegatus*. Early in the investigation the photodynamic effects of the dye neutral red were found to be so striking that it was decided to undertake a study of the effects produced by other dyes. An attempt was made to find concentrations of various dyes which were nontoxic in diffuse light or in darkness but which became toxic during irradiation in sunlight. Eggs were inseminated and then, at selected intervals, transferred to solutions in sea water of the dye being tested, and exposed to sunlight for varied lengths of time.

The problems that presented themselves first concerned effects of different dyes, the effective concentration of these dyes, length of exposure to sunlight necessary to produce toxic effects, wave length effective in the production of photodynamic action, the time of greatest sensitivity of the inseminated egg to the action of the dye, the nature of the result produced, the mechanism of the action, and the effects of temperature on the development of the egg and on the reaction of the egg to the solutions of dye used.

Investigation along these lines was continued for two years; then during 1937 the study of eggs inseminated after irradiation in solutions of dye was begun, and a study of the effects of duration of irradiation and of increased temperature during irradiation was made.

In 1938 the original plan was extended so that it included a study of the effect of intensity of sunlight. A prolonged series of experiments with sunlight of full intensity or, by the introduction of filters between the source of illumination and the eggs, with light of reduced intensity was performed. In 1939 work was continued along the lines of the previous year, chiefly with dyes that had not hitherto been tested.

The dyes used in these experiments included neutral red, picric acid, Martius yellow, anthracene red, chrysoidin R, salicin red B, Janus green B (diazine green), Congo red, alizarin red S, thionin, thiazine red, methylene blue, methylene red, brilliant cresyl blue, resorcin red, Nile blue A, cresyl violet, safranin O, Magdala red echt, nigrosin, auramin O, brilliant green, basic fuchsin, rosolic acid, pyronin, rhodamine B, fluorescein, eosin Y, phloxine, phloxine red, acridine, thioindigo red, phloroglucin-trypan red, madder extract, purpurin-madder, oxamine red, and a few substances such as glutathione, cysteine, cystine, diiodotyrosine, and heparin.

Brief preliminary reports covering the work of each of the years 1935 to 1939 inclusive have been published in Carnegie Institution of Washington Year Books Nos. 34 to 38.

METHODS

Special care was taken to get the sea urchins to the laboratory in the best possible condition. They were placed in glass containers as soon as collected, and during the return to the laboratory they were shaded and the sea water in the containers was changed. When brought in they were used at once.

Each individual was washed in fresh water and cut horizontally through the test, and the aboral part, to which the gonads remain attached, placed upside down in a dry, clean, flat-bottomed finger bowl. In ripe individuals the eggs were at once extruded through the genital pores. Only such individuals were used.

To the eggs in the finger bowls sea water which had been dipped from the ocean at the outer end of the wharf was added. This water was brought into the laboratory in a white enameled container and transferred at once to glass, in order to avoid any possible contamination of the sea water from pumps or piping. The suspended eggs were poured off and after settling were washed twice in sea water. These eggs were used as stock from which controls and experimental cultures were prepared.

Dry sperms were obtained in a similar manner and sperm suspensions in sea water prepared as required. Except on extremely rare occasions when eggs were not abundant, eggs from a single female were used in the experiments. Sperms from a single male were always used in the insemination of the eggs. As standard practice, when inseminated eggs were used in the experiments they were transferred to the solutions of dye 5 minutes after insemination and irradiation was begun at once, that is, 2 minutes before the union of the germ nuclei in fertilization.

A nonirradiated control in sea water and a nonirradiated control in the solution of dye in sea water in diffuse light or in darkness were kept as a check on each irradiated culture.

When the experiments involved the study of eggs inseminated after irradiation, the stock was prepared in the same way. Eggs were transferred from this stock to a finger bowl containing 250 cc. of sea water to serve as control in sea water, and to two similar bowls containing solution of dye in sea water, one of which was kept in diffuse light or in darkness as the nonirradiated dye control, and the other of which was irradiated in sunlight. Immediately after the completion of irradiation this culture was removed to diffuse light and the eggs in the three cultures, namely the control in sea water, the control in the nonirradiated solution of dye, and the irradiated culture, were inseminated with freshly prepared sperm suspension. When more than one irradiated culture was prepared, each additional culture was brought into diffuse light at the end of its period of irradiation and inseminated. Study of the eggs in each culture began immediately after its preparation and was continued intensively on the cultures in rotation until the eggs in the control in sea water had entered the 4-cell stage. After that the cultures were examined at longer intervals.

When it was desired to keep the contents of the finger bowls in darkness, the bowls were surrounded by a closely fitting collar of botanical gray felt drying paper and covered with a small sheet of the same material. When it was desired to irradiate the contents of the bowl through a glass filter, a square opening slightly smaller than the filter was cut in this cover and the filter placed over the opening. When Whatman's filter paper was used in reducing the intensity of the light in irradiation or when light filters of sufficient size were obtainable, they were placed directly on the upper edge of the collar of botanical paper. The bowls with their collars and covers were placed on a sheet of drying paper.

The cytological study of the eggs demanded the use of fixed and stained material. For general use Bouin's picro-formol (Merck's picric acid, saturated aqueous solution, 75 parts; Merck's neutral formaldehyde, 25 parts; Merck's glacial acetic acid, 5 parts) was used. For special purposes Champy, Flemming, Nassanov, and sublimate acetic were used. The eggs were embedded in paraffin, were cut into 5-micron sections, and were stained by the Heidenhain iron haematoxylin process. When a counterstain seemed desirable, erythrosin was used.

Early in the investigation, because of the lack of uniformity of staining of the irradiated eggs, stirring during the process of irradiation was begun. This practice was continued throughout the investigation. Theoretically, if the photodynamic effect is due to the formation of a photocompound of the dye, this should have no effect. Practically it resulted in a uniform reaction and effect. Obviously when irradiation was through filters a means of stirring without admission of unfiltered light had to be devised. For this purpose glass stirrers mounted on a glass pin attached to the bottom of the finger bowl with paraffin and operated by revolving bar magnets were used in part of the work (fig. 1).

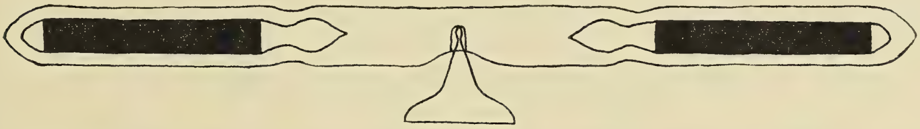

Fig. 1. Glass magnetic stirrer: side view, showing glass pivot supporting rotor made of tubing in which two soft iron rods are enclosed.

During most of the work, however, when the eggs were irradiated through filters they were not stirred during irradiation. When the layer of eggs on the bottom of the dish was sufficiently thin, stirring during irradiation resulted in no increase in the number of cytolized eggs.

It was found necessary in some instances to prevent excessive rise of temperature during irradiation. This was accomplished by stirring the cultures with test tubes containing ice or ice water, keeping meantime a constant thermometric check on the cultures. This obviously could not be done during irradiation through filters, but when filters were used the temperature in the irradiated dishes did not become excessive.

EXPERIMENTS WITH NEUTRAL RED

Procedure

The work of 1935, 1936, and part of 1937 was done on inseminated eggs. The work of the latter part of 1937 and all of 1938 and 1939 was done on uninseminated eggs. The neutral red used in the experiments was from four sources: one lot from the Coleman and Bell Company, one lot from the National Aniline and Chemical Company (both of these certified by the Commission on Standardization of Biological Stains), and two different lots of Grübler, designated in this paper as I and II. Grübler II was used only during four days in 1939, and Grübler I was used in all other work of 1935 to 1939 inclusive. Some preliminary work was done on the concentration of solutions of neutral red, and the concentration of 1:150,000 in sea water was found satisfactory.

After the preliminary work showed that neutral red had a photodynamic effect on fertilized sea-urchin eggs, it became desirable to determine the period of greatest sensitivity of the egg. In the first experiment the practice of irradiating the eggs 5 minutes after insemination was begun; this is about 2 minutes before the union of the nuclei. Experiments were performed in which the insemination-irradiation interval was varied; for example, *Lytechinus* eggs were inseminated at 9:22 A.M. and were transferred to solution of neutral red 1:150,000 in sea water as follows:

Control in sea water 9:27. Control beginning normal 2-cell at 9:59, 37 minutes after insemination.

(1) NRSW,[1] diffuse light, 9:27. Beginning 2-cell at 10:00.

(2) NRSW, irradiated and stirred 9:27–9:37. 99 per cent blister cytolysis, began 10:07.

(3) NRSW, irradiated and stirred 9:32–9:42. 50 per cent blister cytolysis, began 10:07; 10 per cent irregular division.

(4) NRSW, irradiated and stirred 9:37–9:47. 30 per cent blister cytolysis, began 10:07; 15 per cent poor division.

(5) NRSW, irradiated and stirred 9:42–9:52. 30 per cent blister cytolysis, began 10:10; 20 per cent approximating normal division.

(6) NRSW, irradiated and stirred 9:47–9:57. 20 per cent blister cytolysis, began 10:17; 20 per cent approximating normal division.

(7) NRSW, irradiated and stirred 9:52–10:02. 20 per cent blister cytolysis, began 10:30; 50 per cent approximating normal division, entering 2-cell at 10:10, 48 minutes after insemination; 30 per cent abnormal division.

(8) NRSW, irradiated and stirred 9:57–10:07. 50 per cent divided, entering 2-cell at 10:11, 49 minutes after insemination; about half of these 2's blistered.

The results of this treatment were of two kinds, the production of blister cytolysis and the inhibition or modification of cell division. The controls developed normally. The results showed that the eggs were most sensitive when irradiated 5 minutes after insemination and that they became progressively less sensitive during the next 35 minutes.

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[1] Solution of neutral red in sea water.

In another experiment *Lytechinus* eggs were inseminated in sea water at 9:35 and were transferred to solution of neutral red 1:150,000 in sea water 5 minutes after insemination and at 10-minute intervals thereafter until 10:10, each fraction being irradiated for 10 minutes after transfer. The results were similar to those just described with the exception that complete cytolysis resulted in all the irradiated cultures. No normal cleavage beyond the 4-cell stage occurred. The experiments showed that the effects of an irradiation of 10 minutes varied with the phase of nuclear division, irradiation of this length being most effective when it was begun before the union of the germ nuclei in fertilization. Similar results followed other experiments of the same type. In most instances complete cytolysis ultimately followed irradiation.

Another series of experiments for the purpose of determining the effect of duration of irradiation was performed. The experiments of August 1, 1935, will serve as an example. *Lytechinus* eggs were inseminated at 9:54. At 9:59 they were transferred to the control in sea water and to solutions of neutral red 1:150,000 in sea water and treated as follows:

(1) NRSW in diffuse light
(2) NRSW irradiated 1 minute
(3) NRSW irradiated 2 minutes
(4) NRSW irradiated 3 minutes
(5) NRSW irradiated 4 minutes
(6) NRSW irradiated 5 minutes
(7) NRSW irradiated 6 minutes
(8) NRSW irradiated 7 minutes
(9) NRSW irradiated 8 minutes
(10) NRSW irradiated 9 minutes
(11) NRSW irradiated 10 minutes

Irradiation up to and including 3 minutes produced no visible effects, the first cleavage occurring normally. Irradiation of 4, 5, and 6 minutes was followed by the formation of normal 2-cell stages, but blisters were formed in the cleavage furrows. Irradiation of 7 minutes was followed by the formation of poor 2-cell stages with blisters in the cleavage furrows. Irradiation of 8 minutes was followed by a few irregular divisions with rare blistering. Irradiation of 9 and 10 minutes was followed by no divisions, and about 50 per cent of the eggs were completely blistered. Later observation of these eggs showed that when the eggs in the dye control in diffuse light were in a normal 32-cell stage, about 2 hours after insemination, those in the culture that had been irradiated 1 or 2 minutes were in the 16-cell stage; those that had been irradiated 3 minutes were in the 8- or 16-cell stage; those that had been irradiated 4 minutes were in the 8-cell stage. The eggs in the cultures which had received 1, 2, 3, and 4 minutes irradiation were all slightly irregular. Eggs in the cultures irradiated 5 minutes and longer differed from these. Those irradiated 5 minutes formed irregular 4- and 8-cell stages; those irradiated 6 minutes formed irregular 1-, 2-, 4-, and 8-cell stages; those irradiated 7 minutes formed irregular 1-, 2-, and 4-cell stages; those irradiated 8 minutes formed irregular 1-, 2-, and 4-cell stages; most of those irradiated 9 minutes were completely cytolized; all those irradiated 10 minutes were cytolized. In later experiments, after an irradiation of 12 minutes there were no normal divisions; a few eggs divided abnormally but cytolysis followed. Of eggs irradiated 15 minutes, 10 per cent remained undivided; 90 per cent showed complete cytolysis. Finally, after irradiations of 20 and 25 minutes, during which the temperature rose to 34.2° C. and 35.7° C. respectively, there was complete blister cytolysis. This result raised the question whether the effect was due in part to heat cytolysis.

The question of the effect of irradiation with sunlight on *Lytechinus* eggs had arisen earlier and had been answered by the results of an experiment in which the following setup was used:

(*a*) Control in sea water, kept in diffuse light, temperature 28° C.
(*b*) Control irradiated 10 minutes, beginning 11 minutes after insemination, temperature at end of irradiation 29.2° C.
(1) NRSW 1:150,000, kept in diffuse light, temperature 28° C.
(2) NRSW 1:150,000, irradiated 10 minutes, beginning 11 minutes after insemination, temperature at end of irradiation 29.2° C.

The control, (*a*), 38 minutes after insemination showed a few slight cleavage furrows. The irradiated control, (*b*), 38 minutes after insemination showed many eggs with cleavage furrows and some in the 2-cell stage. Both of these cultures 3 minutes later or 41 minutes after insemination showed many eggs in the 2-cell stage. Many of the eggs in the neutral red control, (1), kept in diffuse light, were in the 2-cell stage 39 minutes after insemination; none of the eggs in the irradiated neutral red sea water, (2), had divided or showed cleavage furrows 39 minutes after insemination. Most of the eggs in (1) had divided 46 minutes after insemination; 1 in 50 of the eggs in (2) showed cleavage furrows 56 minutes after insemination. When the eggs in (1) were in the 4- or 8-cell stage, 50 per cent of the eggs in (2) were in a poor 2-cell stage and 50 per cent had failed to divide. The experiment showed clearly that irradiation in sunlight (*b*) with the temperature rising to 29.2° C. produced no harmful effect.

On August 2, 1935, two additional experiments without neutral red for the determination of the effect of sunlight and increased temperature were performed.

1. Eggs 5 minutes after insemination at a temperature of 29.5° C. were irradiated and stirred for 20 minutes, during which the temperature of the sea water rose to 34.2° C., and were then brought back into diffuse light. The blastomeres were slightly wrinkled; they were entering a normal 2-cell stage 48 minutes and a normal 4-cell stage 82 minutes after insemination. The development of the control was also slow.

2. Eggs 5 minutes after insemination at a temperature of 29.5° C. were irradiated and stirred for 25 minutes, during which the temperature of the sea water rose to 34.4° C., and were then brought back into diffuse light. These eggs were entering the 2-cell stage, the blastomeres of some of the eggs being slightly wrinkled, 35 minutes after insemination; they were entering the 4-cell stage 59 minutes after insemination. This insemination-cleavage interval was the same as in the control in sea water in diffuse light.

Subsequent work showed that although injury to eggs irradiated in sea water at these high temperatures was reversible and the eggs so treated developed normally to gastrulae and plutei, irradiation in solutions of dyes at temperatures above 32° C. produced results that must be attributed to excessive temperature. The effect in these cases is not alone due to the photodynamic effect of the dye, but is to be regarded as the effect of the dye plus that of the increased temperature.

Eggs with intact nuclei when irradiated in solutions of neutral red underwent blister cytolysis, the surface of the egg forming a mass of colorless blisters while the center remained as a red core containing the nucleus. If placed in neutral red solutions and irradiated after the nucleus had broken down and the mitotic spindle formed, the eggs completed this division but later development was abnormal.

COMPARISON OF EFFECTS OF IRRADIATION ON EGGS WITH A RESTING NUCLEUS AND A NUCLEUS IN MITOSIS

Most of the investigations described above were made on eggs that were still in the 1-cell stage when irradiated. A series of investigations was planned in which eggs were used that had been inseminated and allowed to develop to 2-cell stages or later before irradiation. In eggs that were transferred to neutral red and irradiated during late anaphase, cleavage was distinctly slower than in nonirradiated neutral sea water. The cultures contained 8- and 16-cell stages when those in the nonirradiated neutral red control were in 32- and 64-cell stages. The results indicated that although division was retarded by irradiation, the cells were less affected by irradiation in solution of neutral red in sea water when they were in active division. This being true, an experiment was planned to show the effect on eggs in the 2-cell stage when they were transferred to solution of neutral red in sea water with the nucleus in a resting stage. Such eggs were transferred to 1:150,000 solution of neutral red in sea water and irradiated for 10 minutes. The contours of these eggs became irregular and the nuclei in 2-cell and 4-cell stages moved to the periphery of the cell. The eggs disintegrated in the 4-cell stage. In another experiment eggs that were in the 2-cell stage, most of them with resting nuclei but some in their second anaphase, were transferred to 1:150,000 solution of neutral red in sea water and irradiated for 10 minutes. In these eggs during the 2- and 4-cell stages the nuclei moved out to the periphery of the blastomeres. Some of the eggs proceeded with cleavage to irregular 12- and 16-cell stages.

FILTERS, 1937

After it was found that *Lytechinus* eggs showed the photodynamic effects of neutral red so well, a preliminary investigation to determine the effective wave lengths of the spectrum was made, and experiments were begun in which such filters as were available were tried. The concentration of neutral red used throughout the work done with filters was 1:150,000 in sea water.

1. The first filter used was a Wratten A filter no. 25, which transmits infrared and red light. Eggs were transferred 5 minutes after insemination to

(1) NRSW, diffuse light, temperature 29.4° C.
(2) NRSW, irradiated 10 minutes, temperature then 33.2° C.
(3) NRSW, irradiated 10 minutes through Wratten A filter no. 25, temperature then 31.2° C.

The eggs in (1), like the eggs in the control in sea water, had developed to the 4-cell stage 56 minutes after insemination; the same was true of the eggs in (3), i.e., those irradiated through the Wratten A filter. The eggs in (2), on the other hand, were all blister cytolized within 56 minutes after insemination. The question arose, Did the eggs in (3) develop normally because of the lower temperature of 31.2° C. or because the Wratten A filter absorbed the injurious wave lengths of light? Two years later the effect of reduced intensity of the light used during irradiation became a part of the question.

2. In another experiment eggs were transferred 3 minutes after insemination to

(1) NRSW, diffuse light, temperature 29.2° C.
(2) NRSW, irradiated 20 minutes.
(3) NRSW, irradiated 20 minutes through Wratten A filter no. 25.

The eggs in (1) cleaved normally and gave rise to fine normal plutei. Those in (2) all showed irregular contours 28 minutes after insemination, and some showed complete blister cytolysis at this time. Development went no farther in this culture. The eggs in (3) showed slightly irregular contours 29 minutes after insemination, but all segmented well and gave rise to uniform normal plutei.

3. Experiments were then begun to show the effect of removal of the ultraviolet light from the sunlight used in irradiation. An empty finger bowl was used as a filter. Eggs were transferred 5 minutes after insemination to

(1) NRSW, 29.4° C, irradiated 20 minutes, temperature then 33.8° C.
(2) NRSW, irradiated 20 minutes through finger-bowl filter, temperature then 33.2° C.

The eggs in (1) 4 minutes after the end of irradiation showed irregular contours and 2 minutes later showed the formation of blisters. All the eggs showed blister cytolysis 11 minutes after the end of irradiation. The eggs in the (2) culture, on the other hand, 4 minutes after the end of irradiation showed smooth contours; 11 minutes after the end of irradiation the contours were still smooth; 16 minutes after the end of irradiation a few of these eggs were entering the 2-cell stage; 5 minutes later the blastomeres of some of these eggs were blistering slightly. The divisions were few and the blastomeres were blistering when formed. These eggs formed abnormal 16- to 32-cell stages but cytolysis was rare.

4. Eggs were transferred 5 minutes after insemination to

(1) NRSW, irradiated and stirred 20 minutes, temperature then 34.2° C.
(2) NRSW, irradiated 20 minutes through finger bowl half filled with sea water, temperature then 32.8° C.

(3) NRSW, irradiated 20 minutes through Wratten K-2 filter, temperature then 32.6° C.

The apparent color of the K-2 filter is yellow. It absorbs the blue and the ultraviolet and transmits infrared, red, and green rays. In (1) blister cytolysis began 36 minutes after insemination; all eggs were beginning to blister 39 minutes after insemination, i.e., 14 minutes after the end of irradiation; all had blister cytolized 49 minutes after the end of irradiation. In (2) the contours of the eggs became slightly irregular 9 minutes after the end of irradiation; all eggs had irregular contours and some were blistering 14 minutes after the end of irradiation; some were blister cytolizing and some were irregular 2-cell stages 18 minutes after the end of irradiation; 10 per cent of the eggs had cytolized, some were in the 2-cell stage, and there were a few 4-cell stages 71 minutes after the end of irradiation. In (3) the contours of all eggs were smooth 10 minutes after the end of irradiation; there were some normal 2-cell stages and no cytolysis had appeared 19 minutes after the end of irradiation; most of the eggs were in nearly normal 2-cell stages 22 minutes after the end of irradiation; these eggs were entering the 4-cell stage, as were the eggs of the control, 62 minutes after insemination. They proceeded normally to the 8-cell stage, none cytolizing.

5. A Wratten K-1 filter was then used. Eggs were transferred 5 minutes after insemination to a solution of neutral red in sea water and irradiated through a Wratten K-1 filter for 20 minutes. The eggs were stained pale pink, and most of them had a smooth contour, but some were becoming irregular 1 minute after the end of irradiation. Neutral red granules were visible and the contour of most of the eggs was slightly irregular 11 minutes after the end of irradiation. These eggs were entering a normal 2-cell stage 15 minutes after the end of irradiation or 40 minutes after insemination. They continued normal cleavage in tempo with the control in sea water. Development in both the control and the experimental culture had proceeded to the swimming blastula stage 3 hours and 35 minutes after insemination. The apparent color of this filter is very light yellow. It absorbs some of the ultraviolet and some blue-violet rays.

6. In the experiments of August 4, 1935, eggs were transferred 5 minutes after insemination to

(1) NRSW, irradiated and stirred 20 minutes, temperature then 32° C.
(2) NRSW, irradiated 20 minutes through finger bowl of sea water, temperature then 32° C.
(3) NRSW, irradiated 20 minutes through cobalt glass filter, temperature then 31.6° C.

In (1) there was complete blister cytolysis; blistering began 11 minutes after the end of irradiation; nearly all the eggs were blistered 16 minutes after the end of irradiation; blister cytolysis had become complete 76 minutes after the end of irradiation. In (2) the contours of the eggs had become irregular 6 minutes after the end of irradiation; nearly all the eggs were blistered 16 minutes after the end of irradiation; there were a few irregular 2-cell stages but most of the eggs had cytolized 35 minutes after the end of irradiation; blister cytolysis had become complete 2 hours and 21 minutes after the end of irradiation. In (3) the eggs were less red than in (2) at the end of irradiation; the contours of these eggs had become slightly irregular 7 minutes after the end of irradiation; the eggs were entering the 2-cell stage 37 minutes after insemination, the insemination-cleavage interval being about 1 minute shorter than in the control, and the temperature in (3) being 2.4° C. higher at the end of irradiation than in the control. After the return of this culture to the laboratory the cleavage in these eggs became somewhat slower than in the control, the eggs of the experimental culture being in 8- and 16-cell stages when those of the control were in 16- and 32-cell stages. The cells of the cleaving eggs in (3) were loaded with large neutral red granules. At 6¾ hours after insemination, the culture contained merely clumps of cells, whereas the control contained blastulae.

7. Eggs were transferred 5 minutes after insemination to

(1) NRSW, irradiated and stirred 10 minutes, temperature then 32.6° C.
(2) NRSW, irradiated 10 minutes through green glass filter, temperature then 32.4° C.
(3) NRSW, irradiated 10 minutes through thin blue glass filter, temperature then 32.8° C.

In (1) the contours of the eggs were becoming irregular and blistering was beginning 23 minutes after the end of irradiation; blistering had increased 30 minutes after the end of irradiation. In (2) the eggs were entering the 2-cell stage 35 minutes after insemination, as in the control; there were some 2-cell stages in which the blastomeres became slightly irregular. The eggs in (3) were entering the 2-cell stage 35 minutes after insemination and cleaved regularly in tempo with the control and (2) to the 8- and 12-cell stage, which they had reached 1 hour and 43 minutes after insemination. The eggs of the control continued normal development, whereas those of (2) and (3) 5¾ hours after insemination had become clumps of abnormal cells. The early saving effect of the green glass and blue glass filters is probably due to the reduction of intensity of irradiation when they are used.

8. Eggs were transferred 5 minutes after insemination to solution of neutral red in sea water and irradiated for 18 minutes through a sheet of common window glass. These eggs had irregular contours 1 minute after the end of irradiation. Their color was pale brown. The eggs had become pinker and the pinkest ones had begun to blister 6 minutes after the end of irradiation. All had very irregular contours and cytolysis was increasing 7 minutes after the end of irradiation. Cytolysis was almost complete 24 minutes after the end of irradiation, when the control eggs were entering a normal 2-cell stage, 47 minutes after insemination. Most of the experimental eggs cytolized completely; a few died in the 2-cell stage. The latter were red; the cytolized eggs lost their color.

9. Eggs were transferred 5 minutes after insemination to

(1) NRSW, 30° C., irradiated and stirred for 20 minutes, temperature then 36.4° C.
(2) NRSW, 30° C., irradiated 20 minutes through Spencer daylight glass filter, temperature then 34° C.

The eggs in (1) were beginning to blister 10 minutes after the end of irradiation; above 90 per cent were blistered 23 minutes after the end of irradiation; all were blistered, no divisions having occurred, 43 minutes after the end of irradiation. The eggs in (2) were less deeply stained than in (1) 5 minutes after the end of irradiation; many had become irregular in form 10 minutes after the end of irradiation; a few were elongating before division to irregular 2-cell stages, covered with blisters, 18 minutes after the end of irradiation. These eggs formed irregular 4-cell stages and a very few bad 8-cell stages, but developed no farther. It should be noted that the temperature in both of these cultures was excessive. It should be noted further that irradiation through the Spencer daylight glass was effective despite the considerable reduction in intensity of irradiation.

Summary of Experiments with Filters

1. Wratten A filter no. 25; apparent color red; transmits infrared and red; absorbs green, blue, and ultraviolet. No cytolysis; culture followed to the 8-cell stage. Temperature 31.2° C.

2. Wratten A filter no. 25; apparent color red; transmits infrared and red; absorbs green, blue, and ultraviolet. No cytolysis; all segmented and developed well to excellent uniform plutei.

3. Empty finger bowl used as screen to absorb ultraviolet. The eggs blistered. The temperature reached during the experiment, 33.2° C., was excessive.

4. Finger bowl half filled with sea water used as screen to absorb ultraviolet. Some eggs blistered. The temperature reached during the experiment, 32.8° C., was slightly excessive.

Wratten K-2 filter; apparent color yellow; transmits infrared, red, and green; absorbs blue and ultraviolet. No cytolysis; culture followed to swimming-blastula stage. The temperature reached during the experiment, 32.6° C., was slightly excessive.

5. Wratten K-1 filter; apparent color very light yellow; transmits red, yellow, green, and some blue; absorbs some ultraviolet and some blue-violet. Normal cleavage, normal blastulae which were swimming 3 hours and 35 minutes after insemination.

6. Finger bowl filled with sea water used as screen to absorb ultraviolet. Nearly all eggs blistered and cytolized. Temperature 32° C.

Cobalt glass filter. Irregular 8- to 16-cell stages, then clumps of cells. Temperature 31.6° C.

7. Green glass filter. Normal 8- to 12-cell stages; finally clumps of abnormal cells and a few abnormal blastulae. The temperature reached during the experiment, 32.4° C., was slightly excessive.

Thin blue glass filter. Normal 8- to 12-cell stages; finally clumps of cells, no living blastulae. The temperature reached during the experiment, 32.8° C., was slightly excessive.

8. Common window-glass filter; absorbs ultraviolet. Most eggs cytolized completely; a few cleaved to 2-cell stage.

9. Spencer daylight glass filter. Slight blistering, irregular 4- and 8-cell stages; developed no farther. The temperature reached during the experiment, 34° C., was excessive.

The experiments described above show conclusively that the effective light in the production of the photodynamic effect of neutral red lies in the short waves of the visible spectrum. Cultures protected by a Wratten A filter no. 25 and irradiated with sunlight developed perfectly, and those that were screened from ultraviolet light alone were killed by an exposure of 20 minutes to sunlight. It will be noted that when the temperature exceeded 32° C., mention is made of that fact in the description. No conclusions have been drawn from experiments in which the temperature was excessive.

The work described above was done before the study of effects of intensity of irradiation was begun. In 1938 several Corning glass color filters were used in experiments. The results of these experiments will be described later in this paper (see pp. 22–27). They confirm the conclusions reached during the preliminary work. They emphasize, however, the fact that, because of the variability in the amount of light transmitted, intensity of irradiation must also be taken into consideration. The transmission of visible light by some of the filters is so low that the intensity of the light transmitted is insufficient to produce photodynamic reactions.

The work with color filters was discontinued when the conclusion was reached that the effects of wave lengths could be studied to better advantage with a monochromator.

Reactions of the Egg to Neutral Red and to Irradiation in Solutions of Neutral Red

It was found that when inseminated *Lytechinus* eggs were transferred to 1:150,000 solution of neutral red in sea water and kept in diffuse light they usually passed through a series of changes in color from brownish pink to deep red. They became brownish pink in 1 to 4 minutes, pink in 5 to 9 minutes, and red in 15 to 25 minutes. The color described is at first in the cytoplasm, which stains uniformly. In these eggs neutral red granules became visible in 8 to 16 minutes, the granules, scattered uniformly throughout the egg, first stained lightly, then deeply. Some eggs required a longer time, staining in these not reaching its highest intensity in less than 25 minutes.

Inseminated eggs transferred to solution of neutral red in sea water and then irradiated in sunlight became brownish pink in 3 to 5 minutes, pink in 9 to 16 minutes, and red in 11 to 20 minutes; a few became red in as little as 2 minutes. The neutral red granules in these eggs varied greatly in the time of their appearance. In some eggs they were visible in as little as 2 minutes, in others they did not reach their fullest intensity of color until 31 minutes after the beginning of irradiation.

In the blastomeres of dividing eggs the free periphery of the cell is especially free from neutral red granules, although there may be rare scattered granules in this region. The peripheries along the line of contact between adjacent blastomeres are less free from granules, but as the blastomeres round up these also become freer. The granule-free region is at first colorless and transparent. Later it becomes pink.

Irradiation of late 4-cell stages that had developed from eggs transferred 5 minutes after insemination to solution of neutral red in sea water and kept in diffuse light did not interfere with division to the 8-cell stage. It resulted in the cytoplasm's acquiring a distinct yellowish-pink color, in movement of the nuclei to the outer edge of the blastomeres, and in development of irregularity of contour in the blastomeres, succeeded by complete blister cytolysis in many eggs. Development went no farther than the formation of irregular 16-cell stages. Micromeres were not formed. The effect of irradiation of late 4-cell stages in solution of neutral red was thus shown to be of the same character as in the case of undivided eggs.

It was found, as stated above, that when the neutral red granules first appeared they were distributed uniformly throughout the cytoplasm. In eggs kept in solution of neutral red in sea water in diffuse light they then became aggregated around the nucleus, leaving the peripheral cytoplasm free from granules. In similar eggs in solution of neutral red in sea water that had been irradiated in sunlight this concentration of neutral red granules was less rapid. The aggregation of granules became much more striking after the mitotic figures were formed, the concentration becoming so great that it seemed, as was suggested by Fischel (1901), that it must interfere with the processes of division. After the daughter nuclei were formed, the neutral red granules again became rather uniformly distributed throughout the egg. They again became concentrated around the intact nucleus just before the next division, and became concentrated once more around the mitotic figures in this division. This rhythmical distribution of granules was characteristic and followed the succeeding cycles of division.

Pre-irradiation versus Post-irradiation

Early in the experiments the idea of the formation of a photocompound of the dye by sunlight irradiation was considered.

1. NRSW 1:150,000 was irradiated for 10 minutes and the effect of this solution was compared with the effect of NRSW 1:150,000 irradiated in sunlight for 10 minutes after the eggs had been placed in it. Eggs were placed in these solutions 10 minutes after insemination. The eggs placed in the pre-irradiated solution developed with the same appearance and in the same tempo as those in the nonirradiated NRSW kept in the dark, eggs in both entering the 2-cell stage 37 minutes after insemination. The eggs in these solutions developed in the same tempo as those in the control in sea water. Eggs in the NRSW irradiated for 10 minutes after the eggs had been placed in it lagged perceptibly in their cleavage. The contours of the blastomeres were bad.

2. Irradiated NRSW 1:150,000 was prepared by placing a chemical flask

(pyrex 1000 cc.) containing the solution in the sun for 2 hours. The color faded from reddish brown to light yellow, the temperature when the flask was brought in being 49.3° C. On the following day eggs were transferred 5 minutes after insemination to this pre-irradiated solution (2) and their development compared with that of eggs in NRSW 1:150,000 in diffuse light (1) and with that of eggs placed in pre-irradiated NRSW 1:150,000 five minutes after insemination and irradiated and stirred for 10 minutes (3). The eggs in (2) developed in the same tempo as those in the control in sea water and in (1). The 2-cell stage was for a time slightly irregular in form, but subsequently became smooth. Later, however, these eggs formed rather poor 4-, 8-, and 12-cell stages. Some of them remained undivided. The eggs placed in pre-irradiated NRSW and then irradiated (3) showed irregular 2-cell stages and some blister cytolysis of undivided eggs. When the eggs of the control and of the cultures in diffuse light were in the 32-cell stage, the post-irradiated eggs (3), like those of the pre-irradiated (2) culture, were poor 4's, 8's, and 12's. When the eggs of the control and of the diffuse-light neutral red cultures had developed to blastulae beginning to swim, 5 hours and 27 minutes after insemination, those of both the pre-irradiated and post-irradiated cultures were all cytolizing. The eggs in these cultures did not go beyond the 12-cell stage. It is therefore evident that the pre-irradiated neutral red was effective in checking cleavage and producing cytolysis. The second irradiation produced no additional effect.

3. Eggs 5 minutes after insemination were placed in (1) nonirradiated NRSW, temperature 27.4° C., and (2) 2-hour-irradiated NRSW, temperature 27.8° C. The first 2-cell stages appeared in both cultures 41 minutes after insemination. Cleavage proceeded regularly and on the following morning all (control, [1], and [2]) contained fine young plutei. The pre-irradiated neutral red had no effect. Possibly it was too old, as it had been prepared 10 days before. This would mean that the photocompound of the dye had been transformed back into ordinary dye compound.

The pre-irradiated solution had been prepared with the hope that it might be of use on days when sunlight was not available. Because of the unstable nature of the photocompound, this use of the solution proved impossible.

It seems possible that the amount of photocompound of the dye in solution never exceeds a certain amount and that there is a continuous process of formation of photocompound and its change back to the ordinary compound. Continuous light is necessary to maintain the presence of photocompound.

4. Inseminated eggs 5 minutes after insemination were placed in (1) NRSW pre-irradiated for 20 minutes, temperature 29.8° C.; and uninseminated eggs were placed in (2) NRSW pre-irradiated for 20 minutes, temperature 29.8° C., and inseminated at once. The eggs in (1) and its control were entering the 2-cell stage 42 minutes after insemination. The eggs in the control for (2) were entering the 2-cell stage 42 minutes after insemination, and those in (2) were entering the 2-cell stage 57 minutes after insemination. Blister cytolysis appeared in both (1) and (2). Many eggs in (1) were irregular 75 minutes after insemination. Many eggs in (2) were irregular and there was some cytolysis 68 minutes

after insemination. In (1) there were undivided eggs, 2-cell and 4-cell stages, and much blister cytolysis 1 hour and 44 minutes after insemination. In (2) there were undivided eggs, 2-cell and 4-cell stages, and much blister cytolysis 1 hour and 39 minutes after insemination. Cytolysis appeared earlier in eggs that were not inseminated until after they were placed in solution of neutral red in sea water.

The Influence of Temperature on Development

In 1937 a series of experiments was performed in which the influence of temperature during irradiation was ascertained by comparing cooled with non-cooled fractions. The effect of increased duration of irradiation was also studied.

In the first of these, the results were as follows:

Control, 28.4° C., eggs entering 2-cell stage 39 minutes after insemination.

(1) Eggs in NRSW, in diffuse light, 28.4° C., were entering the 2-cell stage 40 minutes after insemination. They continued cleavage normally.

(2) Eggs irradiated 5 minutes in NRSW, cooled to 28.1° C., were entering the 2-cell stage 57 minutes after insemination. They continued to 2's and 4's. All stages blister cytolized.

(3) Eggs irradiated 10 minutes in NRSW, cooled to 28.2° C., had not entered the 2-cell stage 58 minutes after insemination. There was rare cleavage to 2's and 4's. All stages blister cytolized.

(4) Eggs irradiated 15 minutes in NRSW, cooled to 27.9° C., had not divided 59 minutes after insemination and never cleaved. All finally showed blister cytolysis.

The temperature was never excessive. There was progressive increase in the effect of 5-, 10-, and 15-minute irradiation.

In another experiment, the results were as follows:

Control, 28.3° C., eggs entering the 2-cell stage 42 minutes after insemination.

(1) Eggs in NRSW, diffuse light, 28.3° C., were entering the 2-cell stage 44 minutes after insemination and were followed to normal 12's and 16's.

(2) Eggs in NRSW, irradiated 10 minutes, cooled to 28° C. No 2-cell stages had been formed 63 minutes after insemination, when there was increased opacity of the eggs and some were disintegrating. Ten minutes later there had been rare division of some eggs into irregular 2-cell stages and there was some blister cytolysis.

(3) Eggs in NRSW, irradiated 15 minutes, cooled to 28.6° C. There was never any cleavage; 20 per cent of the eggs blister cytolized, many became irregular in form and died, and the surface on about 1 per cent became rough.

(4) Eggs in NRSW, irradiated 15 minutes, not cooled, 33.6° C. There was never any cleavage; a definite irregularity of the surface appeared 13 minutes after the end of irradiation and many eggs were blistered 4 minutes later. Within 40 minutes after the end of irradiation 90 per cent had blis-

tered; some of these eggs cytolized, with the red color diffusing into the cytoplasm. Above 90 per cent of these eggs blister cytolized; the remainder were surface roughened and undivided.

Here again there was progressive increase in the effect of 10- and 15-minute irradiation in the cooled cultures, and the violence of the reaction increased in cultures in which the temperature rose to 33.6° C.

In another experiment the eggs in the control, in (1) the diffuse-light culture, and in (2) the culture cooled during an irradiation of 10 minutes were not inseminated until 11 minutes after the respective treatments.

Control, 27.8° C., many eggs in 2-cell stage 41 minutes after insemination.

(1) Diffuse light, 27.8° C., some eggs were beginning to divide 48 minutes after insemination and were followed to irregular 4's.

(2) Culture irradiated and cooled for 10 minutes, 27.9° C., the eggs being inseminated 1 minute after the end of irradiation. No fertilization membranes were formed and there were one or two blisters on each egg 3 minutes after the end of irradiation. In a few of these eggs the surface roughened and lifted away from the red core. Fifty-seven minutes after insemination there was a high degree of blister cytolysis, and 13 minutes later, or 70 minutes after insemination, the blisters were beginning to break off from the surface of the egg and float away. The end result in this culture was complete cytolysis.

(3) Culture irradiated and cooled for 15 minutes, 27.4° C., the eggs being inseminated at the end of irradiation. No fertilization membranes were separated. The surface of the eggs became rough and lifted away from the red core 22 minutes after the end of irradiation. The surface of most of the eggs then became highly blistered, some of the blisters becoming extremely large. The blisters began to break off from the surface of the egg and to float away 50 minutes after the end of irradiation. They had the appearance of large spherical or irregular vesicles. The end of the process was complete cytolysis.

(4) The eggs were irradiated for 15 minutes, not being cooled during the process, and reached a temperature of 32.6° C. They were inseminated at the end of irradiation and formed no fertilization membranes. The surface of some of the eggs became rough 8 minutes after insemination. During the next 15 minutes the surface of all became rough, and 5 minutes later had lifted away from the deeply stained red central core in all the eggs. The eggs had completely cytolized 3¾ hours after the end of irradiation.

In all the irradiated eggs, except those in (4) (which did not form blisters), the blisters dropped off leaving a red core. Here there was little difference in result between the eggs cooled during an irradiation of 10 minutes and those cooled and irradiated for 15 minutes, the only difference lying in the somewhat greater violence of the reaction following the irradiation of 15 minutes. In (4), in which irradiation was at a temperature 0.6° C. above the temperature maximum, the reaction was so violent that blisters were not formed, but the entire surface lifted from the red center of the egg and the stain began to diffuse into areas which were otherwise clear.

The experiments in this group that have thus far been described were with irradiations lasting 5, 10, and 15 minutes. The effects of irradiations of 20 minutes were then considered.

In the first of these experiments the following protocol was used:

Control in sea water, 29.8° C.
(1) Eggs in NRSW, diffuse light, 10 minutes, observed temperature 29.8° C.
(2) Eggs in NRSW, irradiated 10 minutes, cooled, observed temperature 29.7° C.
(3) Eggs in NRSW, irradiated 20 minutes, cooled, observed temperature 29.7° C.
(4) Eggs in NRSW, irradiated 20 minutes, observed temperature 31.6° C.

The eggs of the control, of (1), and of (2) were inseminated as soon as the 10-minute irradiation of (2) was completed. The fertilization membranes in the control were well separated, in (1) were poorly separated, and in (2) were not separated. The eggs in (3) and (4) were inseminated as soon as their irradiation was completed. No fertilization membranes were separated in either culture. The eggs in the control showed the first cleavage furrow 40 minutes after insemination, as did the eggs in (1). There was no indication of division in the eggs of (2) 42 minutes after insemination, and blister cytolysis of the eggs had begun. In the eggs in (3) and (4) blister cytolysis had begun 27 minutes after insemination and had increased to 30 per cent 15 minutes later. At 82 minutes after insemination, the eggs in the control were in the 4- and 8-cell stage; those in (1) were in the 4-cell stage; those in (2) showed no divisions and some were in blister cytolysis. At 72 minutes after insemination the eggs in (3) showed no divisions and 30 per cent blister cytolysis, and those in (4) showed no divisions but increased cytolysis. The 20-minute irradiation of these eggs did not result in so complete a cytolysis as did the 15-minute irradiation of the previous experiment. It did, however, result in the death of all the eggs. It seemed desirable, therefore, to repeat the experiment with an irradiation of 20 minutes.

In the next experiment the protocol was

Control in sea water, 28.7° C.
(1) Eggs in NRSW, diffuse light, 28.7° C., 20 minutes
(2) Eggs in NRSW, irradiated 20 minutes, cooled to 28.8° C.
(3) Eggs in NRSW, irradiated 20 minutes, held at 32° C.

All the eggs were inseminated 1 minute after the completion of the irradiation of (2) and (3). The eggs of the control and of (1) formed excellent, well separated membranes. Those of (2) and (3) formed no fertilization membranes. Four minutes after insemination many of the eggs in (3) were blistering over the whole surface, and in (2) there were many eggs with a single small blister. Ten minutes after insemination general blistering in (3) had increased to 70 per cent, and in (2) it amounted to 35 per cent. Six minutes later, or 16 minutes after insemination, general blistering in (3) had increased to 100 per cent, and in (2) to 75 per cent; 5 minutes later it was above 90 per cent, and after another 5 minutes, or 26 minutes after insemination, 100 per cent of the eggs in (2) were blistered. The eggs in the control and in (1) began to enter the 2-cell stage 38 minutes after insemination. In (2) and (3) blisters began to break from the red cores 56 minutes after insemination. During the next 10 minutes many blisters broke away from the red-cored eggs, large numbers of clear spheres lying separate and surrounding the red spheres that remained after the separation of the clear vesicles.

The result of this experiment indicated that with an irradiation of 20 minutes the rapidity of the reaction of the eggs was associated with temperature. It seemed clear, because of the difference between the last two experiments and the close resemblance between the results of the last of the 20-minute irradiations and the last of the 15-minute irradiations, that some factor other than temperature and length of irradiation must enter. A possible factor seemed to be the intensity of the light used in irradiation.

Preliminary Experiments with Opal Glass

Pending the opportunity of making a quantitative study of the effects of intensity of light, a short series of qualitative experiments was made using two sheets of milk or opal glass, one marked X, the other unmarked, as filters through which to irradiate the eggs. A spectrophotometric curve of the transmittance of this glass was subsequently obtained.

The results of these experiments follow. The temperatures given are those at the time of insemination.

1. Dull day. All eggs inseminated after (2) and (3) had been irradiated 20 minutes.

Control in sea water, 27.2° C. Followed through late normal cleavage to blastulae.
(1) Eggs in NRSW, in darkness, 27.2° C. Cleaved normally. No blister cytolysis. Followed to blastulae.
(2) Eggs in NRSW, irradiated 20 minutes, 30.6° C. 20 per cent blistered and disintegrated; a few went through 2 divisions; all dead 4¼ hours after end of irradiation.
(3) Eggs in NRSW, irradiated 20 minutes through MG[1] filter X, 28.3° C. Less than 10 per cent with fine blisters; these entered leucocytolytic disintegration; most eggs became blastulae.

2. Bright sun. All eggs inseminated 5 minutes after (2) and (3) had been irradiated 10 minutes.

Control in sea water, 29.4° C. Regular normal cleavage. 30.0° C.
(1) Eggs in NRSW, in darkness, 29.2° C. Regular normal cleavage. 29.9° C.
(2) Eggs in NRSW, irradiated 10 minutes, 32.3° C. Rare fertilization mem-

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[1] Milk glass or opal glass.

branes; blistering 15 minutes after insemination; no division; some leucocytolysis; 16⅔ per cent blistered. 30.0° C.

(3) Eggs in NRSW, irradiated 10 minutes through 2 MG filters, 31.0° C. Regular normal cleavage. 31.0° C.

3. Dull day. All eggs inseminated 4 minutes after (2) and (3) had been irradiated 15 minutes.

Control in sea water, 27.6° C. Regular cleavage to blastulae.

(1) Eggs in NRSW, in darkness, 28.0° C. Regular cleavage to blastulae. No cytolysis.

(2) Eggs in NRSW, irradiated 15 minutes, 29.0° C. All eggs tight fertilization membranes; all eggs irregular, some blister cytolysis 7 minutes after insemination. 20 per cent blister cytolysis 11 minutes after insemination; same 23 minutes after insemination, other eggs less angular than before; some poor division 65 minutes after insemination; all cytolizing 4½ hours after insemination.

(3) Eggs in NRSW, irradiated 15 minutes through MG filter X, 28.7° C. All eggs well separated fertilization membranes; all eggs regular; 3 per cent with fine blisters 20 minutes after insemination; 3 per cent cytolized, most of others to blastulae.

4. Bright sun. Control, (1), and (2) inseminated 6 minutes after end of irradiation of (2). (3) inseminated 6 minutes after end of irradiation of 20 minutes.

Control in sea water, 28.8° C. Normal cleavage, 2's, 4's, 8's.

(1) Eggs in NRSW, in darkness, 28.8° C. Fertilization membranes well separated; normal cleavage 2's, 4's, 8's.

(2) Eggs in NRSW, irradiated 10 minutes, 31.8° C. Fertilization membranes not separated. Blister cytolysis beginning 3 minutes after insemination; 20 per cent blister cytolizing 15 minutes after insemination; no cleavage; blisters dropping off 48 minutes after insemination; all unblistered eggs became rough.

(3) Eggs in NRSW, irradiated 20 minutes through MG filter X, 32.3° C. Fertilization membranes well separated; eggs spherical; 10 per cent blister cytolizing 18 minutes after insemination; all other eggs to 2's and 4's.

5. Bright sun. Control, (1), and (2) inseminated 2 minutes after end of irradiation of (2). (3) inseminated 3 minutes after end of irradiation.

Control in sea water, 27.1° C. Beginning 2-cell 41 minutes after insemination.

(1) Eggs in NRSW, in darkness, 27.4° C. Beginning 2-cell 41 minutes after insemination.

(2) Eggs in NRSW, irradiated 10 minutes, 30.4° C. Slight blistering and irregularity of eggs beginning 17 minutes after insemination; 25 per cent blistered 25 minutes after insemination. All disintegrated 5½ hours after insemination.

(3) Eggs in NRSW, irradiated 30 minutes through MG filter X, 31.4° C. Some

eggs blistering 1 minute after insemination. Unblistered eggs have well separated fertilization membranes 2 minutes after insemination. 10 per cent of eggs blistered 8 minutes after insemination. Beginning 2-cell 43 minutes after insemination. About 50 per cent regular development; 10 per cent blistered eggs disintegrated; remainder irregular development 5½ hours after insemination.

These experiments show the effects of irradiations of different lengths (20, 15, and 10 minutes) without and with the filters, and of short (10-minute) irradiations without the filters compared with longer irradiations (20 and 30 minutes) with the filters.

The results of these preliminary experiments show clearly that reduction of the intensity of irradiation results in reduction of the photodynamic effect.

EXPERIMENTS WITH UNFERTILIZED EGGS

The difference in reaction of eggs that were inseminated before irradiation and eggs that were inseminated after irradiation prompted experiments with irradiation of uninseminated eggs in solution of neutral red in sea water.

In the first of these experiments, eggs in

(1) NRSW, diffuse light, at 28° C. were compared with
(2) NRSW, irradiated 20 minutes, cooled to 27.8° C.

Five minutes after being transferred to the solution of neutral red in sea water the eggs in (1) were lightly stained, while those in (2) were deeply stained. Five minutes later both showed disintegration with considerable blistering. It was not, however, a typical blister cytolysis. The day was somewhat cloudy; the sunlight was strong enough to cast a shadow.

The protocol of the following day was

(1) Eggs in NRSW, diffuse light, 27.8° C.
(2) Eggs in NRSW, irradiated 20 minutes, cooled, inseminated, 28° C.
(3) Eggs in NRSW, irradiated 10 minutes, cooled, not inseminated, 28° C.
(4) Eggs in NRSW, irradiated 20 minutes, cooled, not inseminated, 28.2° C.

The eggs in (1) were smooth and pink 13 minutes after being placed in the solution of neutral red in sea water; those in (3) were smooth and red 14 minutes after their transfer to solution of neutral red in sea water. The eggs in (2) were inseminated at the completion of their irradiation. Four minutes later they were smooth and redder than those in (3), and their fertilization membranes were close, not separated at all. The eggs in (4) 6 minutes after the end of irradiation were smooth, their color being the same as in (2). In (3) 21 minutes after the end of irradiation some eggs showed roughening of the surface and some showed slight blisters. In (4) 12 minutes after the end of irradiation the appearance was about the same as in (3), just noted, but the blisters were more definite. In (1) at this time some of the eggs were irregular in form but none were blistered. In (3), however, at this time, although most of the eggs were smooth, there were some rare eggs with a red core that were blistered, and in (4) 50 per cent of the eggs had a very rough surface and many were badly blistered. Thirty-five minutes after the irradiation of 20 minutes, about 50 per cent of the eggs in (4) were blistered, 10 per cent had a smooth surface, and the remainder were rough. Thirty-five minutes after the end of irradiation lasting 20 minutes and after insemination at the end of irradiation, blistering in (2) was less than in (4) but the surface of nearly all the eggs was rough. Fifty-five minutes after the end of irradiation fully 85 per cent of the eggs in (4) were blistered with the vesicles dropping off, 5 per cent of the eggs were smooth, and 10 per cent had a rough surface. Seventy-seven minutes after the end of irradiation 99 per cent of the eggs of this culture were blistered, lying in a mass of detached vesicles. About 50 per cent of the eggs in (2) at this time were irregularly blistered, most of the remaining eggs being more or less rough. Slightly later there was a gradually increasing amount of leucocytolysis in these eggs, the eggs swelling and the color fading progressively. There was almost no complete blistering such as occurred in (4); there were no detached vesicles. Two and a half hours after the end of irradiation the eggs in the control and in (1) were smooth and spherical, those in (2) had disintegrated, showing leucocytolysis, in (3) less than 10 per cent showed blister cytolysis, the remainder of the eggs being smooth and spherical with some fragments of disintegrating eggs, and in (4) there was complete blister cytolysis.

In both these experiments uninseminated eggs that had been irradiated for 20 minutes in solution of neutral red in sea water with the temperature held to 27.8° C. and 28.0° C. during the process and then inseminated showed atypical blistering, and in (2) of the second experiment they showed swelling and leucocytolysis. It seemed desirable to repeat this experiment with the following protocol:

Control in sea water
(1) Uninseminated eggs in NRSW, in darkness, 28.2° C.
(2) Uninseminated eggs in NRSW, irradiated 10 minutes, 30.6° C.
(3) Uninseminated eggs in NRSW, irradiated 20 minutes, 33.0° C.

The eggs in (1) and (2) were inseminated 11 minutes after their transfer to solution of neutral red in sea water. The control in sea water was inseminated 4 minutes later. The eggs in (3) were inseminated 1 minute after the end of their 20-minute irradiation. Fertilization membranes were well separated on eggs in the control and in (1). No fertilization membranes were formed in (2) and (3). In (3) the surface of all the eggs became rough. Twenty-four minutes after the end of irradiation 10 per cent of the eggs in (2) were blistered. In (3), 14 minutes after the end of irradiation the surface of all the eggs was rough, there was no blistering like that in (2), and leucocytolysis and granular separation of the eggs had begun.

These results confirmed those that had previously been reached on insemination of eggs following an irradiation of 20 minutes.

The Effect of Intensity of Irradiation (Filters, 1938)

Beginning on July 1, 1938, a General Electric exposure meter, with calibrated filters, was used to determine the intensity of sunlight or of artificial light used in irradiating *Lytechinus* eggs in sea water or in solutions of dye in sea water. It seemed desirable to use some simple method of measuring the intensity of sunlight that might be duplicated readily by anyone who wished to do so. The method was first brought to the writer's attention by Professor A. L. Patterson, of the Department of Physics of Bryn Mawr College, and was subsequently developed at Tortugas with the help of Professor Leonard B. Clark, of Union College, who in turn had the benefit of the help and advice of Mr. Frank Benford, of the research laboratories of the General Electric Company at Schenectady, New York.

With a knowledge of the correction necessary for individual meters and of the reciprocals of transmission by any serial combination from one to seven of a series of seven milk-glass filters, it was possible to obtain the intensity of the light used by multiplying the reading in foot-candles given on the face of the meter by the determined factors. For example, the reading was 35 with filters 1 to 5. Multiplying 35 by the factor for the reciprocal of transmission, 284.09, gives 9943.15; then multiplying this by 0.935, the correction factor for the meter used, gives 9296 foot-candles, the actual intensity of light available for irradiation.

Each experimental lot of eggs was divided into portions and the different portions irradiated with light of full intensity or, by the introduction of filters between the source of illumination and the eggs, with light of reduced intensity and different quality.

This method of determining the intensity of irradiation is thoroughly satisfactory on a clear, cloudless day. It is not satisfactory on days when readings vary greatly during a 10- or 15-minute interval. When the variation is not more than 250 it probably is safe to use the average foot-candles as the basis for computation. When it exceeds that amount, any computation save an actual foot-candle-minute one would be undependable. In some observations the figures during a 10- or 15-minute interval ran from 1076 to 8995, from 1535 to 5630, from 2047 to 7102. All figures with a variation of more than 250 have been omitted from the tabulations.

Milk-glass plates, Whatman filter paper no. 50, red glass, and Corning glass filters A.038, 534, 428, 430, 585, and 584 were used in reducing the intensity of irradiation and changing the wave lengths of light reaching the eggs. The percentage of light transmitted by any of these filters varied with the intensity of light used. One milk-glass filter transmits about 42 per cent of the light. Two milk-glass filters transmit about 28 per cent. One sheet of Whatman filter paper no. 50 transmits 57 to 60 per cent of the light, two sheets transmit 38 to 44 per cent, three sheets transmit 28 to 36 per cent. For each experiment, the amount of light transmitted by filter paper was determined. A sheet of red glass used in the experiments transmitted 3 per cent of the light. The figures for transmission of the Corning glass filters were those furnished by the Corning Glass

Works, and were as follows: filter A.038, 86 per cent; filter 534, 50 per cent; filter 428, 24 per cent; filter 430, 8.5 per cent; filter 585, 0.2 per cent; filter 584, less than 0.1 per cent. [The colors of the filters are as follows: A.038, straw yellow (no violet); 428, light blue-green; 430, dark blue-green; 534, light blue (no ultraviolet); 585, dark blue-purple (and ultraviolet); 584, dark red (and ultraviolet). —W. L. D.] See also pages 7–12.

The experiments show that with irradiations of 10 and 15 minutes the threshold for violent surface reaction (blister cytolysis) of *Lytechinus* eggs in 1:150,000 solution of neutral red in sea water lies at about 2500 foot-candles. From this point to about 4300 foot-candles blister cytolysis may increase to as much as 2–15 per cent; it usually stands at about 2 per cent, 15 per cent being unusual. It increases to 20–25 per cent between 7000 and 7500 and to 75–90 per cent between 8000 and 9500 foot-candles. At intensities from 3000 up to 10,000 foot-candles there was a regular increase in the violence of the surface reaction and complete inhibition of the cleavage processes.

It will be noted that in the tables care has been taken to distinguish between the various filters used. With the Corning glass filters, in which the total light transmitted is greatly reduced, the intensity of certain wave lengths is not reduced, and the results with nos. 428 and 430, which remove the noninjurious red but transmit the blue, are striking.

In 1939, neutral red Grübler lot II was used in 1:150,000 solutions. The effect of this dye was not so uniform as that of other lots of Grübler and of National Aniline neutral red.

Comparative studies were made with various lots of dyes and an excellent photographic record of progressive stages of blister cytolysis was made with the assistance of Dr. William L. Doyle. See tabular summary of this work, below.

Tabular Summary

I. Effects of time, intensity, and wave length of irradiation on eggs in solutions of neutral red (1:150,000)

A. *Irradiation time, 15 minutes. Intensity regulated with light filters made of Whatman no. 50 filter paper*

Date (1938)	Light intensity (foot-candles ± 125)	Temperature (°C.)	No. sheets filter paper	Culture no.	Results
7/23	252	28.9	4	(5)	Regular cleavage
7/23	630	29.0	2	(4)	Regular cleavage
7/23	756	29.2	1	(3)	Regular cleavage
7/22	1614	29.1	4	(5)	No blistering; normal cleavage; normal swimming gastrulae

[*Continued on following page*]

A—*Continued*

Date (1938)	Light intensity (foot-candles ± 125)	Temperature (°C.)	No. sheets filter paper	Culture no.	Results
7/23	1616	29.6	0	(2)	No blistering; cleavage abnormal; none normal beyond 8-cell; all cytolized
7/23	1937	30.0	4	(5)	No cytolysis; regular cleavage; prism larvae
7/19	2042	28.7	3	(5)	Blistering less than 1 per cent; excellent regular cleavage
7/10	2147	29.4	3	(4)	Less than 1 per cent blistered; excellent cleavage; excellent blastulae
7/19	2686	28.9	2	(4)	Blistering about 1 per cent; regular 2's, 4's, 32's, 64's
7/23	3228	30.4	2	(4)	No cytolysis; regular cleavage; prism larvae
7/22	3454	29.3	2	(4)	No blistering; normal cleavage; mortality high; some gastrulae
7/10	4300	29.8	1	(3)	4 per cent blistered; abnormal 2's, 4's, 8's; much disintegration
7/22	4301	29.8	1	(3)	No blistering; into 2's; much disintegration
7/19	4301	28.8	1	(3)	2 per cent blistering; somewhat irregular cleavage; 16's to 64's; many irregular
7/7	4304	31.4	0	(2)	15 per cent blister cytolysis; no normal division
7/23	4729	30.6	1	(3)	No cytolysis; irregular cleavage; abnormal gastrulae
7/19	6994	28.9	0	(2)	25 per cent blistered; no cleavage; most disintegrated
7/10	7532	29.9	0	(2)	20 per cent blistered; 2 per cent divided; never beyond 4's; all disintegrated
7/7	7532	30.6	0	(2)	25 per cent blistered; cytolysis in all
7/22	8070	31.6	0	(2)	Blistering; 90 per cent irregular with protruding clear lobes; no division
7/23	8070	32.9	0	(2)	75 per cent cytolysis; no normal division; all died
7/4	9469	33.0	0	(2)	90 per cent blistered; no division

B. *Intensity regulated with opal glass (42 per cent transmission)*

Date (1938)	Light intensity (foot-candles ± 125)	Temperature (°C.)	Time (min.)	No. sheets opal glass	Culture no.	Results
			9- and 10-minute irradiation			
7/8	2109	29.6	10	2	(3)	No blistering; fine young blastulae
7/3	4176	30.4	10	1 X	(3)	2 per cent blistered; 2's and 4's
7/8	7532	30.6	10	0	(2)	10 per cent blister cytolysis; 50 per cent 2's; never beyond 8's; cytolized
7/2	7575	30.9	9	0	(2)	50 per cent blistered; no cleavage
7/1	9469	30.4	10	0	(2)	25 per cent blistered; no 2-cell
7/2	9469	32.4	10	0	(2)	50 per cent blistered; none divided
7/3	9942	32.0	10	0	(2)	55 per cent blistered and disintegrated; none divided
			16- and 20-minute irradiation			
7/1	1075	31.4	16	1 X	(3)	No cytolysis; regular 2's
7/6	2367	27.1	20	0	(2)	Rare blistering; 50 per cent irregular, formless masses of cells; finally all disintegrated
7/2	3182	31.2	20	1 X	(3)	10 per cent blistered; about 50 per cent cleavage

C. *Intensity and wave length regulated with Corning glass filters*

(1) Data arranged according to time of irradiation

Date (1938)	Light intensity (foot-candles)	Temperature (°C.)	Filter no.	Culture no.	Results
			10-minute irradiation		
7/3	20	30.6	585	(4)	1 per cent blistered; 2's and 4's
7/2	805	31.0	430	(3)	5 per cent blistered; good 2's
7/8	1808	30.0	428	(4)	No blistering; normal division to 8's; then broke down
7/1	8143	30.0	A.038	(3)	3 per cent blistered
			15-minute irradiation		
7/3	10	30.2	585	(4)	8 per cent blistered; others 64 plus
7/4	19	30.2	585	(3)	4 per cent blistered; 4's and 8's
7/17	200	30.4	Red glass	(3)	No blistering; 8's, 12's, 16's
7/7	1033	30.6	428	(4)	2 per cent blister cytolysis and 2's, some of which blistered; all disintegrated
7/7	2152	31.4	534	(3)	10 per cent blister cytolysis; no normal division; disintegration
7/4	2273	30.6	428	(4)	20 per cent blistered; 4's and 8's
			20-minute irradiation		
7/2	9	32.3	584	(4)	No blistering; 4's and 8's
7/6	201	27.4	430	(4)	Blister cytolysis less than 1 per cent; 99 per cent divided; about 1 per cent normal development
7/6	568	27.4	428	(3)	Blister cytolysis less than 1 per cent; 99 per cent divided; about 1 per cent normal development
7/7	640	30.6	430	(4)	10 per cent blistered; no cleavage beyond 12-cell; cytolysis in 25 per cent of cleaved and uncleaved eggs
7/7	1808	30.8	428	(3)	10 per cent blistered; no cleavage beyond 4-, 8-, 12-cell; cytolysis in about 25 per cent of cleaved and uncleaved eggs
			30-minute irradiation		
7/18	17	28.6	430	(4)	No blistering; regular cleavage; blastulae; plutei

(2) Data arranged according to wave lengths transmitted

Date (1938)	Light intensity (foot-candles)	Temperature (°C.)	Time (min.)	Culture no.	Results
				Filter no. 584	
7/2	9	32.3	20	(4)	No blistering; 4's and 8's
				Filter no. 585	
7/3	20	30.6	10	(4)	1 per cent blistered; 2's and 4's
7/3	10	30.2	15	(4)	8 per cent blistered; others 64 plus
7/4	19	30.2	15	(3)	4 per cent blistered; 4's and 8's
				Filter no. 534	
7/7	2152	31.4	15	(3)	10 per cent blister cytolysis; no normal division; disintegration
				Filter no. 428	
7/8	1808	30.0	10	(4)	No blistering; normal division to 8's; then broke down
7/7	1033	30.6	15	(4)	2 per cent blister cytolysis and 2's, some of which blistered; all disintegrated
7/4	2273	30.6	15	(4)	20 per cent blistered; 4's and 8's
7/6	568	27.4	20	(3)	Blister cytolysis less than 1 per cent; 99 per cent divided; about 1 per cent normal development
7/7	1808	30.8	20	(3)	10 per cent blistered; no cleavage beyond 4-, 8-, 12-cell; cytolysis in about 25 per cent of cleaved and uncleaved eggs
				Filter no. 430	
7/2	805	31.0	10	(3)	5 per cent blistered; good 2's
7/6	201	27.4	20	(4)	Blister cytolysis less than 1 per cent; 99 per cent divided; about 1 per cent normal development
7/7	640	30.6	20	(4)	10 per cent blistered; no cleavage beyond 12-cell; cytolysis in 25 per cent of cleaved and uncleaved eggs
7/18	17	28.6	30	(4)	No blistering; regular cleavage; blastulae; plutei
				Filter no. A.038	
7/1	8143	30.0	10	(3)	3 per cent blistered
				Red glass	
7/17	200	30.4	15	(3)	No blistering; 8's, 12's, 16's

II. Effects of time and intensity of irradiation (in direct sunlight) on post-inseminated eggs in neutral red (Grübler lots II and I) solutions (1:150,000)

A. *Grübler lot II*

Date (1939)	Culture no.	Light intensity (foot-candles)	Temperature (°C.)	Time (min.)
6/30	(2)	4516–5047	29.1	10
	(3)	4516–5047	29.4	15
7/1	(2)	9297–9562	30.3	10
	(3)	9297–9562	31.2	15
7/3	(2)	8234	30.3	10
	(3)	8234	30.2	15
7/4	(2)	9031–9297	30.9	10
	(3)	9031–9297	30.9	15

Results. 6/30 (2). Membranes poorly separated 13 minutes after insemination; (1) membranes well separated 2 minutes after insemination; (1) dividing 39 minutes after insemination; (2) no cleavage 54 minutes after insemination; eggs divided later irregularly to 4's, 8's, 12's, 16's; on following morning all dead and disintegrated.

6/30 (3). 2 minutes after insemination an occasional separated fertilization membrane; 3 minutes after insemination more than 50 per cent irregular outline; 16 minutes after insemination surface of eggs smooth; 35 minutes after insemination above 90 per cent with narrowly separated fertilization membranes, less than 1 per cent blister cytolysis; 49 minutes after insemination no cleavage; 1 hour 48 minutes after insemination irregular division to 2's and 4's; day following, all dead and disintegrated.

7/1 (2). 7 minutes after insemination no fertilization membranes; 17 minutes after insemination eggs smooth, deep red, no vacuoles; 25 minutes after insemination a few eggs rough, infrequent blister cytolysis; (1) 43 minutes after insemination, into 2's; (2) not yet constricting; 4 hours after insemination 85 to 90 per cent cytolysis and disintegration; on the following morning completely disintegrated.

7/1 (3). 1 minute after insemination no fertilization membranes, many vacuoles; 11 minutes after insemination vacuolization less evident, eggs deep red; 17 minutes after insemination less than 1 per cent blister cytolysis; 2 minutes later increased blister cytolysis, also vacuoles with inclusions; 38 minutes after insemination 10 per cent blister cytolysis; 4 hours after insemination 100 per cent blister cytolysis and disintegration; on following morning completely disintegrated.

7/3 (2). 2 minutes after insemination no separated membranes, some pitting; 23 minutes after insemination no blisters; control into 2's 39 minutes after insemination; (1) into 2's 43 minutes after insemination; (2) no constrictions 44 minutes after insemination; no eggs divided during 9 hours.

7/3 (3). 3 minutes after insemination no separated membranes; pitting; 18 minutes after insemination blisters beginning; 41 minutes after insemination no constrictions, no typical blister cytolysis, more in nature of lobular cytolysis; during 9 hours no eggs divided, many became colorless.

7/4 (2). 3 minutes after insemination no separated membranes; 10 minutes after insemination a few poorly separated close membranes; (1) 36 minutes after insemination first constriction; (2) 37 minutes after insemination no constriction, a little blister cytolysis; control, 2's and into 2's 40 minutes after insemination; (2) 42 minutes after insemination none dividing.

7/4 (3). 3 minutes after insemination no separated membranes; 22 minutes after insemination surface of eggs beginning to roughen; 33 minutes after insemination much lobular cytolysis; 39 minutes after insemination none dividing, some blister cytolysis, more lobular cytolysis. See plate 1, figure 1.

B. *Grübler lot 1*

Date (1939)	Culture no.	Light intensity (foot-candles)	Temperature (°C.)	Time (min.)
7/5	(2)	7305	30.1	10
	(3)	7305	30.0	15
7/22	(2)	5578	29.9	12

Results. 7/5 (2). No separated membranes 2 minutes after insemination; 18 minutes after insemination about 1 per cent pitting, less than 1 per cent blister cytolysis; 59 minutes after insemination blisters separating; 6 hours after insemination disintegration complete. See plate 1, figures 4, 5; plate 2, figures 1, 5.

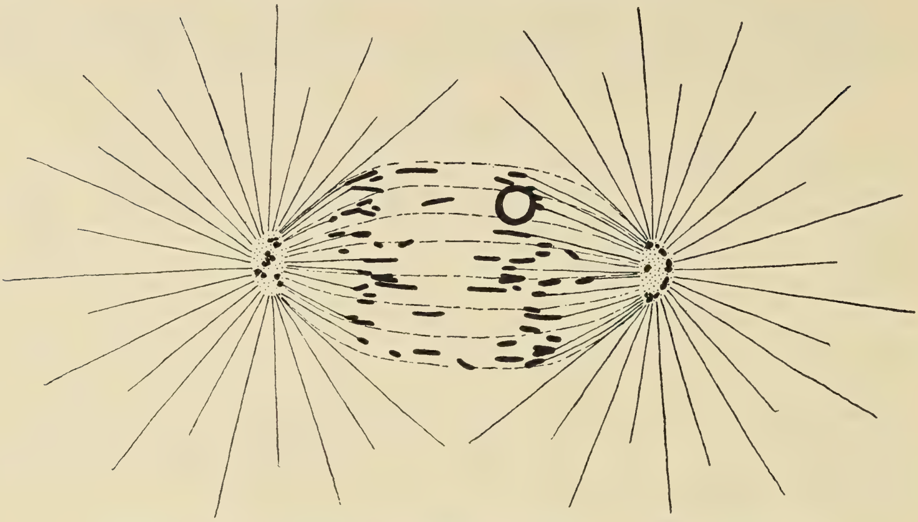
7/5 (3). 3 minutes after insemination no separated membranes, some pitting, less than 1 per cent; 12 minutes after insemination eggs with slightly rough surface, some blister cytolysis beginning; 6 hours later disintegration complete. See plate 1, figures 2, 3; plate 2, figures 2, 6. For control see plate 1, figure 6; plate 2, figures 3, 4.

7/22 (2). 3 minutes after insemination no separated fertilization membranes; control and (1) beginning 2's 41 minutes after insemination; (2) no 2's, 5 per cent blister cytolized 39 minutes after insemination; (2) 99 minutes after insemination no 2's, cytolysis increased to 15 per cent.

Results of the Study of Sections of Eggs from Neutral Red Cultures

Miss Marion Bridgman studied the cytology of inseminated nonirradiated and irradiated *Lytechinus* eggs in solutions of neutral red. These eggs were transferred to their respective solutions 10 minutes after insemination. One set was kept in diffuse daylight while the other was irradiated in sunlight for 5 minutes and then brought back into diffuse daylight. In the nonirradiated set dark granules appeared in the asters and in the late anaphase were visible in the equatorial plate. The spindle fibers were shorter than in the control, and the chromosomes showed a slight lagging in their division. In eggs fixed in late anaphase these modifications had become more apparent and there was a concentration of neutral red granules in and about the spindle. In the resting stage they were dispersed evenly throughout the cell but moved in at the time of mitosis.

In the irradiated eggs the effects were similar but more marked. During prophase the neutral red granules were scattered throughout the cell, but by late anaphase dark granules had appeared in the asters and also in the equatorial plate. The astral rays were shorter than in the nonirradiated eggs and there was the same lagging in the distribution of the chromosomes. As the eggs proceeded to metaphase the neutral red granules could be seen pressing in around the spindle and becoming heavily concentrated about the equatorial plate. During



Figures 2 to 40 are drawings of sectioned material. Legends give dye used, date of experiment, and time of fixation.

FIG. 2. Neutral red, 7/28/35, 10:26 A.M.

anaphases the chromosomes were more irregular in their division and several remained in the region of the equatorial plate until the others had almost reached the poles of the spindle.

It is thus evident that neutral red in itself has a considerable effect on mitosis, an effect that is increased when the cell is irradiated.

The study of slides prepared from other fixations confirms Miss Bridgman's observations. Irradiations of 5, 10, 15, and 20 minutes were performed. The temperature was permitted to rise in one instance to 35.7° C. Increase of irradiation to 20 minutes with the temperature held below 30° C. resulted in inhibition of cleavage and complete blister cytolysis. This blister cytolysis came on rather gradually, becoming complete 26 minutes after the end of irradiation. When the temperature was allowed to rise to 32° C. blistering was complete 16 minutes

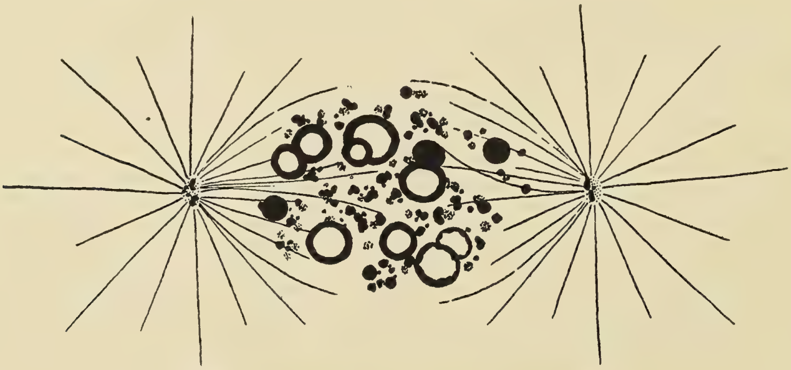


FIG. 3. Neutral red, 7/28/35, 10:26 A.M.

after the end of irradiation. With irradiation of 15 minutes at a temperature of 30° C., 50 per cent of the eggs were blistered and were disintegrating 37 minutes after the end of irradiation. With the same duration and at lower temperatures (fig. 2) there was less disintegration but increased lagging of chromosomes. With an irradiation of 10 minutes, the temperature held at 30.6° C., there was about 10 per cent blister cytolysis an hour after the end of irradiation. With an irradiation of 15 minutes and reduction of intensity to 3686–4177 foot-candles there was division with greatly increased lagging and spherules on the spindle (fig. 3). With irradiation of 15 minutes and reduction of intensity to 2459–2787 foot-candles blister cytolysis was reduced to 5 per cent, there was little lagging, and there were some spherules on the spindle.

The results show that duration of irradiation beyond 5 minutes results in increased lagging. This increased lagging is closely associated with an increase in intensity of irradiation and an increase in temperature.

THE EFFECT OF SUNLIGHT ON UNSTAINED EGGS

Very early in this investigation the possibility was considered that irradiation with sunlight affected the development of *Lytechinus* eggs in sea water. The possible effect of increase in temperature was also considered. From time to time experiments dealing with these phases of the investigation have been performed.

In the first of these experiments, the cleavage of inseminated eggs in an irradiated culture was compared with that in a control culture kept in diffuse light at 28° C. A few of the control eggs showed very slight constriction 38 minutes after insemination. Most of them were in the 2-cell stage 48 minutes after insemination. They were well into the 4-cell stage in 64 minutes after insemination. Eggs from the same insemination irradiated with sunlight in sea water for 10 minutes, at the end of which the temperature had reached 29.2° C., were compared with this culture. Their development was somewhat more rapid than that of the eggs in the control. Some had entered the 2-cell stage and many had begun constriction 38 minutes after insemination. The difference in tempo between control and irradiated culture was less obvious after the first division. The reason for this lies in the fact that the temperature of the irradiated culture soon dropped to that of the diffuse-light culture when the two were kept side by side in diffuse light. The cultures were indistinguishable 60 minutes after insemination.

Another experiment on unstained eggs was made to test the effect of sunlight irradiation with a greater rise in temperature. The control consisted of *Lytechinus* eggs transferred from the sea water in which they were inseminated to fresh sea water at a temperature of 29.3° C. 8 minutes after insemination. These eggs were spherical and smooth and began to enter the 2-cell stage 36 minutes after insemination. They continued cleavage regularly. Eggs of the same insemination, transferred to fresh sea water 8 minutes after insemination, were irradiated and stirred for 10 minutes. At the close of irradiation the temperature of the culture was 32.3° C. These eggs remained spherical and smooth. They began to enter the 2-cell stage 36 minutes after insemination. Their further cleavage was excellent and showed no abnormalities. Similarly, eggs of the same insemination were transferred from the sea water in which they had been inseminated to fresh sea water 8 minutes after insemination and irradiated and stirred for 20 minutes. At the end of irradiation the temperature of this culture had risen to 34.8° C. The eggs of this culture were smooth and spherical at the close of irradiation. They began to divide, somewhat irregularly, to 2-cell stages 37 minutes after insemination. Most of these eggs had divided, their blastomeres being somewhat irregular, 14 minutes later. In the culture an hour after insemination there were many undivided eggs, irregular in form but not blister cytolized, and many irregular 2-cell stages; some of the 2-cell stages were of normal appearance. Most of the eggs of this culture had become smooth and were in the 2- or 4-cell stage 90 minutes after insemination. They continued their cleavage regularly and on the following morning had reached a gastrula stage normal in appearance and behavior, but not quite so far advanced as those from the culture that had been irradiated only 10 minutes and had been subjected to a temperature of 32.3° C. It should be noted that the eggs recovered from the effects of this temperature, 34.8° C., reached during irradiation in sea water.

These results are essentially similar to those obtained from experiments about a week later. In the first of these the control consisted of inseminated eggs kept in diffuse-light culture at 28.6° C. The surface of these eggs became slightly rough shortly after insemination. This condition gradually improved. Both rough and smooth eggs began to enter the 2-cell stage 37 minutes after insemination. Within 7 minutes nearly all had become smooth 2-cell stages although a few were still in anaphase. They continued their development regularly and normally and 8 hours after insemination were normal swimming blastulae. Eggs of the same insemination, transferred to sea water, were irradiated and stirred for 20 minutes. At the end of this time the temperature of the culture had risen to 33.3° C. The eggs were then of about the same degree of roughness as those of the control. They also began to lose their roughness about 30 minutes after insemination and were all in or going into the 2-cell stage, some still being in anaphase, 42 minutes after insemination. They continued cleavage normally, although there were many irregularities during the 8-cell stage. They had become blastulae with mesenchyme 8 hours after insemination. Sunlight irradiation and a temperature of 33.3° C. did not have any marked effect in this experiment. Even the roughening of the surface that one associates with heat cytolysis occurred in the control kept at a normal temperature in diffuse light.

We may compare with this the results of an experiment performed on the following day with the same duration of irradiation and very nearly the same range of temperature. The control consisted of inseminated eggs kept in sea water in diffuse light at a temperature of 28.8° C. The eggs were smooth and were in anaphase 36 minutes after insemination. They entered a smooth 2-cell stage regularly and were followed for 2 hours, at the end of which they had reached the normal 16-cell stage. Eggs of the same insemination were transferred to fresh sea water 5 minutes after insemination and irradiated and stirred for 20 minutes. A temperature of 33.3° C. was reached at the end of irradiation. The eggs were then somewhat indented but 5 minutes later had become smooth. They divided to 2-cell stages which were not quite smooth. Their development was followed for an hour, when the eggs were in the 2- and 4-cell stages, precisely like those of the control at that time. No cytolysis appeared in these eggs.

Finally we will note the results of an experiment in which the range of temperature was somewhat less than in the experiments just described and the duration of irradiation 15 instead of 20 minutes. The eggs of the control in diffuse light at 29.2° C. were slightly irregular in form from the time of insemination until about 35 minutes after insemination, when they became smooth and began to enter the 2-cell stage. These eggs continued their cleavage normally and had reached a late cleavage stage with a large blastocoele 3 hours after insemination. Eggs of the same insemination were transferred to fresh sea water

5 minutes after insemination and irradiated and stirred for 15 minutes. During irradiation the temperature rose to 32.4° C. These eggs were irregular in form at the close of irradiation. Like those of the control, they became smooth and were entering the 2-cell stage 38 minutes after insemination. The eggs of this culture kept pace with those of the control throughout early cleavage and 3 hours later were in a late cleavage stage with a large blastocoele.

These experiments show very conclusively that exposure to a temperature of 34.8° C. during irradiation in sea water does not result in an irreversible injury to the eggs. It will be found, however, that irradiation in some of the solutions of dye in sea water at temperatures above 32° C. results in injury from which the eggs cannot recover.

EXPERIMENTS WITH OTHER DYES

Picric Acid

Picric acid was used as a dye in concentrations varying from a saturated solution to a 1:100,000 solution in sea water. Possibly because of its use in fixing fluids, picric acid is commonly regarded as highly toxic to living cells. It was therefore with some surprise that it was noted that fertilized *Lytechinus* eggs developed well in solutions as strong as 1:4000.

The first concentration used was a 1:100,000 solution of picric acid (Merck reagent) in sea water. The eggs were placed in the solution 5 minutes after insemination, temperature 28.4° C. One portion of the eggs was irradiated and stirred for 13 minutes, the temperature rising 3.2° C. during the irradiation. The irradiated eggs divided regularly, development being more rapid than in eggs of either the unstained or the dye control. The insemination-cleavage interval of the irradiated eggs was 33 minutes; of the sea-water control, 34 minutes; and of the dye control, 36 minutes. The eggs were allowed to remain in their respective solutions and all—i.e., sea-water control, nonirradiated dye control, and irradiated eggs—gave rise to excellent plutei with skeletal rods. The only departure from the normal modal condition lay in the fact that the body rods were not united in the posterior part of the body. This was true of the control as well as of the experimental plutei.

A 1:50,000 solution of picric acid (Merck reagent) was then tried. Fertilized *Lytechinus* eggs were placed in 1:50,000 solution of picric acid in sea water, temperature 28.8° C., 5 minutes after insemination. One portion of the eggs was irradiated with sunlight and stirred for 12 minutes. The temperature rose 3.2° C. during this interval. Again the insemination-cleavage interval in the irradiated eggs was shortened; the eggs in this portion began cleavage 34 minutes after insemination, the eggs in the dye control and the eggs in the sea-water control 1 to 2 minutes later. Here again the later development was normal, the eggs in sea water, in the dye control, and in the irradiated solutions giving rise to excellent plutei whose posterior end was angular, not rounded, and whose skeletal rods were good, although again not united in the posterior part of the body.

The next concentration tried was 1:20,000. Five minutes after insemination, eggs were placed in solution of picric acid, temperature 29.1° C., and one portion irradiated and stirred for 12 minutes; during the irradiation the temperature rose 2.9° C. Again the insemination-cleavage interval was shorter in the irradiated culture than in the control and in the dye control, the difference in time being 1 minute. Development in all cultures was good and was followed as far as the formation of late gastrulae and prism larvae.

A concentration of 1:10,000 was then tried. *Lytechinus* eggs 5 minutes after insemination were transferred to 1:10,000 solution of picric acid in sea water, temperature 28.6° C. One portion was irradiated and stirred for 10 minutes, the temperature rising 1.7° C. during the treatment. Again there was excellent normal cleavage in all the cultures. The insemination-cleavage interval was 1 to 2 minutes shorter in the irradiated eggs than in either of the controls.

It will be noted that in all the instances in which the insemination-cleavage interval was shorter in the irradiated eggs than in the controls, the temperature in the irradiated cultures was 1.7° to 3.2° C. higher than in the controls.

In the continuation of this investigation, inseminated *Lytechinus* eggs were irradiated in increasingly stronger solutions of picric acid in sea water, up to saturation. In a 1:8000 solution, temperature 28.7° C., in which inseminated eggs were placed 5 minutes after insemination, irradiated, and stirred for 20 minutes, and whose temperature was kept equal to that of the controls, the insemination-cleavage interval was 39 minutes, slightly (1 minute) longer than in the controls. Development was followed only to the 4-cell stage, the first 2 cleavages being normal.

In a 1:6000 solution of picric acid in sea water, temperature 28.7° C., eggs 5 minutes after insemination were irradiated and stirred for 20 minutes, the temperature during this time rising 0.3° C. above that of the diffuse-light control. The insemination-cleavage interval was 40 minutes in all the cultures. Early cleavage was normal.

Eggs placed in a 1:4000 solution of picric acid in sea water, temperature 28.7° C., 5 minutes after insemination, and irradiated and stirred for 20 minutes, the temperature dropping 0.2° C. below that of the diffuse-light culture, cleaved normally; some eggs, however, failed to divide. The insemination-cleavage interval was 43 minutes and longer in all the cultures.

Eggs placed in a 1:2000 solution of picric acid in sea water, temperature 28.7° C., 5 minutes after insemination, and irradiated and stirred for 20 minutes, the temperature being 0.2° C. lower than in the diffuse-light control, failed to divide, the eggs remaining spherical with intact nuclei.

Eggs 5 minutes after insemination were transferred to 1:1000 solution of picric acid in sea water, temperature 29.9° C., and irradiated and stirred 10 minutes, during which the temperature rose 0.9° C. In both the diffuse-light control and the irradiated culture, the entire surface of the eggs became covered with fine blisters, the irradiated eggs being yellower and more blistered than those in the nonirradiated control. There was no cleavage.

The experiment was concluded by irradiating and stirring eggs for 10 minutes in saturated solution of picric acid in sea water, temperature 30° C. In the nonirradiated control all the eggs were dead within 2 minutes after being placed in the picric acid solution. Their surface was smooth and their appearance opaque. The eggs in the irradiated cultures were dead and were very dark in color at the end of irradiation.

In another experiment, a 1:10,000 concentration of picric acid in sea water, temperature 28° C., was used. Uninseminated *Lytechinus* eggs were irradiated and stirred for 20 minutes, the temperature being controlled and varying from the control by only 0.2° C. The eggs in both the control and the irradiated culture were inseminated 21 minutes after being placed in the solutions of dye. In both, fertilization membranes were only slightly and incompletely separated from the surface of the eggs as late as 10 minutes after insemination. The insemination-cleavage interval was long, 53 minutes. Cleavage was followed to the 16-cell stage; it progressed slowly, the cells failing to round up in the characteristic way and remaining somewhat compressed against each other. There was no blistering and no disintegration.

In the experiments just considered, the eggs were subjected to the action of picric acid either after or before insemination. In the eggs inseminated before treatment, the fertilization membranes were well separated from the surface of the eggs and the picric acid apparently did not pass through this membrane. The eggs developed to all appearances as well in the sea-water solution of picric acid as in sea water. In the eggs inseminated after irradiation in solution of picric acid, the fertilization membranes were less well separated from the surface of the eggs and the insemination-cleavage interval was long. There was still no evidence that picric acid had entered the egg.

In the first set of experiments it was not until a concentration of 1:2000 was reached that the eggs failed to divide; even at the last-mentioned concentration there was no evidence of the entrance of picric acid into the egg. In the 1:1000 solution of picric acid in sea water the surface of the eggs became covered with fine blisters, and the cytoplasm became yellower in the irradiated eggs than in the control.

From the evidence furnished by the experiments it is possible to conclude that the initial effect of picric acid was on the surface of the eggs. Until the surface was modified, picric acid did not enter the eggs.

The experiments described above show clearly that picric acid has at most a limited photodynamic effect. The conclusion is stated in this form because of the slight difference in behavior of the irradiated and nonirradiated cultures in 1:1000 solution of picric acid in sea water.

Results of the Study of Sections of Eggs from Picric Acid Cultures

The development of inseminated *Lytechinus* eggs irradiated in solutions of picric acid was uniform and normal. No difference in appearance between eggs irradiated for 20 minutes in 1:4000 and for 12 minutes in 1:20,000 solution was noted. The same statement may be made concerning eggs irradiated for 20 minutes in 1:10,000 solution. The agreement between all these eggs was closer than that between samples of eggs in the 1:10,000 solution fixed 6 minutes apart. In those fixed earlier the astral rays reached the surface of the egg; in those fixed later the asters were bushy and few rays reached the surface.

MARTIUS YELLOW

The Coleman and Bell Company's Martius yellow was used in these experiments. More dilute solutions having proved ineffective, a saturated sea-water solution of the dye, about 1:2000, was used. Inseminated *Lytechinus* eggs were transferred to 1:2000 solution of Martius yellow in sea water 5 minutes after insemination and kept in diffuse light at a temperature of 29.9° C. The eggs were stained slightly yellow within 2 minutes after being placed in the dye; 5 minutes later there had been no increase in color. The eggs remained smooth until about an hour after fertilization, when many eggs became indented. About an equal number of eggs remained smooth and undivided; a very few had divided to irregular 2-cell stages. Most of the eggs of this culture were still undivided an hour and a half after insemination. The culture finally contained some 2- and some 4-cell stages as well as some irregular and some cytolized eggs. The dye in this concentration was evidently toxic.

In another diffuse-light culture in saturated solution of Martius yellow in sea water at 28.8° C., most of the eggs became superficially roughened within 15 minutes after being placed in the solution of dye. About half of these eggs were still rough with intact nuclei 45 minutes later. Within the next 4½ hours all the rough eggs had cytolized, while the smooth eggs had undergone no development. Here again, saturated solution of Martius yellow in sea water was shown to be toxic in diffuse-light cultures.

With each of these diffuse-light cultures an irradiated culture may be compared. For the first comparison, eggs were transferred 5 minutes after insemination to 1:2000 solution of Martius yellow in sea water and irradiated and stirred for 10 minutes. The temperature then stood at 32.7° C. These eggs showed a yellow color of the same intensity as those in the diffuse-light culture. For comparison with the second diffuse-light culture, eggs were transferred 5 minutes after insemination to 1:2000 solution of Martius yellow in sea water and irradiated and stirred for 11 minutes. The temperature stood at 30.6° C. at the close of irradiation. The eggs of the culture were then slightly rougher than those of the diffuse-light culture. When about half the eggs of the diffuse-light culture were rough with intact nuclei, at least three-quarters of the irradiated eggs had become rough and indented with intact nuclei. Four and a half hours later, this culture was the same as the nonirradiated culture. All the rough eggs had cytolized and the smooth eggs showed no development. Irradiation with sunlight seems to have had no effect on the activity of the dye. In both the diffuse-light and the irradiated cultures the dye was toxic. The irradiated cultures showed no increased toxicity.

Cultures were then studied in which 1:4000 solution of Martius yellow in sea water was used. Eggs were transferred 5 minutes after insemination to 1:4000 solution of Martius yellow in sea water and kept in diffuse light. Many of these eggs were badly indented 37 minutes after insemination. At this time the eggs of the sea-water control were beginning to enter the 2-cell stage. It was not until 25 minutes later that an occasional 2-cell stage was found in the diffuse-light culture. The culture at this time also contained some cytolized, undivided eggs and many badly indented eggs. This culture, an hour and a half after insemination, contained some 2-cell and 4-cell stages together with many irregular and some cytolized eggs. The cleavage stages were distinctly abnormal. Still later, 2 hours after insemination, scattered 2-cell, 4-cell, and 8-cell stages were present; most of the undivided eggs were indented and many were cytolized. The development in this more dilute sea-water solution of Martius yellow was far from normal, but it was distinctly better than that in the stronger solution. With this culture there may be compared a culture made by transferring eggs of the same insemination to 1:4000 solution of Martius yellow in sea water 5 minutes after insemination. These eggs were irradiated and stirred for 10 minutes. The culture contained, 2 hours after insemination, scattered and irregular 2-cell, 4-cell, and an occasional 8-cell stage. Most of the eggs were indented and undivided and many were cytolized.

The diffuse-light culture and the irradiated culture were therefore in essential agreement. The dye on sunlight irradiation showed no photodynamic effect.

Results of the Study of Sections of Eggs from Martius Yellow Cultures

In nonirradiated eggs in solution of Martius yellow there were black globules in contact with the nuclear membranes. These large black globules were usually opposite each other in the cytoplasm, at "12 and 6 o'clock," "1 and 7 o'clock," "4 and 10 o'clock," etc. There were many egg and sperm nuclei in contact (fig. 4).

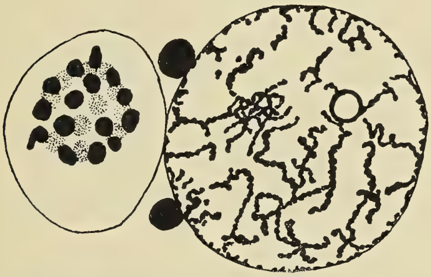


FIG. 4. Martius yellow, 8/3/36, 12:04 P.M.

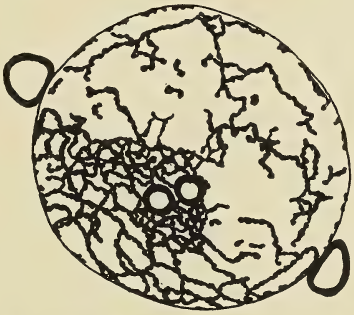


FIG. 5. Martius yellow, 8/3/36, 12:05 P.M.

In irradiated eggs, deeply stained spherules appeared on the egg nucleus, usually opposite each other (fig. 5). There were male and female nuclei in contact. These eggs were essentially in agreement with the nonirradiated eggs.

The globules which appeared in both cultures are probably without significance.

ANTHRACENE RED

Anthracene red was used in solutions of less than 1:20,000 strength. Fertilizations were poor in the irradiated solutions, 20 per cent of the eggs showing irregular cleavage and 80 per cent remaining undivided.

CHRYSOIDIN R

Chrysoidin R, Grübler, was used in 1:50,000 solution. Well separated fertilization membranes were formed on both nonirradiated and irradiated eggs. Cleavage was normal, but slower than in the sea-water control. Later development was also slow.

The following table gives a brief summary of the experiments.

Summary of Results of Irradiation of Eggs in Solutions of Chrysoidin R 1:50,000

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
6/29/39............	(1)	28.2	1:50,000	0	Dark
	(2)	28.9	1:50,000	10	5977
	(3)	30.1	1:50,000	15	5977

Results. (1) Post-inseminated; eggs rather deeply stained yellow within 2 minutes; within 5 minutes very yellow; surface of eggs wrinkled meridionally; well separated thin membranes 3 minutes after insemination; control into 2's 39 minutes after insemination; (1) none constricting; a few beginning to elongate and constrict 45 minutes after insemination; increased constriction and division during the next 4 minutes; constriction from one pole of egg; many had divided, others were dividing 51 minutes after insemination; 3 hours and 24 minutes after insemination 8's and 16's irregular; controls in 32's; on the following morning all were dead.

(2) Post-inseminated; well separated thin membranes 3 minutes after insemination; none constricting 40 minutes after insemination; 46 minutes after insemination rare elongation and constriction, mainly from one pole; 52 minutes after insemination much unipolar constriction, some bipolar constriction; 3 hours and 24 minutes after insemination irregular 8's and 16's; on the following morning rotating blastulae.

(3) Post-inseminated; 2 minutes after insemination well separated thin membranes; 35 minutes after insemination none constricting; 47 minutes after insemination constriction, mainly unipolar, begun; 3 hours and 18 minutes after insemination irregular 8's and 16's; on the following morning rotating blastulae.

SALICIN RED B

Salicin red B, Kahlbaum, formed an excellent distilled-water solution which was not miscible with sea water.

JANUS GREEN B (DIAZINE GREEN)

The use of Janus green B is of especial interest because of the common use of this dye for intra vitam staining of mitochondria.

Janus green from two sources was used. One was a Hoechst preparation; the other was diazine green, National Aniline and Chemical Company, total dye content 64 per cent, certified. Both these dyes were found to be highly toxic in all concentrations used up to 1:800,000. All the results reported here were obtained with the National Aniline Company dye unless otherwise stated. With concentrations from 1:50,000 down to 1:300,000 the dye was lethal to inseminated *Lytechinus* eggs even in cultures in diffuse light. In concentrations of 1:50,000, eggs in cultures in diffuse light showed blue granules in the cytoplasm, and they failed to cleave and finally disintegrated. In inseminated eggs kept in 1:100,000 solutions in sea water in diffuse light, granules in the peripheral zone were stained, but no cell divisions occurred. The reaction of eggs in 1:200,000 solutions in diffuse light was the same except that the peripheral granules were less intensely stained. Eggs in 1:300,000 solutions in sea water kept in diffuse light became irregular in outline, and showed heavily stained granules and very occasional division to the 2-cell stage. The results were the same in 1:400,000 solutions.

The effect of irradiation with sunlight on eggs in solutions in sea water was not especially marked until 1:300,000 and 1:400,000 solutions were reached. At these concentrations no cleavage whatever appeared in the irradiated cultures. Eggs in the more concentrated 1:100,000 solution became slightly bluer than those in 1:200,000, but in both were so blue that the nucleus could be seen only with difficulty.

Early in this work it became evident that stock solutions of Janus green undergo deterioration quickly. In order to get consistent results from day to day, freshly prepared solutions must be used (Tennent, 1936).

Inseminated *Lytechinus* eggs, transferred to 1:500,000 Janus green 5 minutes after insemination and kept in diffuse light, showed bluish granules throughout the egg within 15 minutes. Some of these eggs divided to 2-cell stages 44 minutes after insemination, being about 7 minutes slower than those of the sea-water control. Cleavage in these eggs continued somewhat irregularly to 4- and 8-cell stages during the time that the eggs of the control were reaching the 32-cell stage. Inseminated eggs, transferred to 1:500,000 Janus green in sea water 5 minutes after insemination, and irradiated and stirred for 20 minutes, showed greenish cytoplasm with bluish granules scattered throughout the egg. The granules were somewhat difficult to see. The granules gradually began concentration toward the center of the egg, leaving the periphery clear, about 5 minutes after the end of irradiation. These eggs showed much cytolysis but no cleavage during the remainder of their development.

Inseminated *Lytechinus* eggs, transferred to 1:600,000 solution of Janus green in sea water in diffuse light 5 minutes after insemination, showed bluish granules scattered throughout the egg within 25 minutes. Most of these eggs divided regularly to 2- and 4-cell stages; some divided irregularly. They continued their division to the 8-cell stage, many of the cleaving eggs being normal but some abnormal. Inseminated *Lytechinus* eggs transferred to 1:600,000 Janus green 5 minutes after insemination, and irradiated and stirred for 20 minutes, showed greenish cytoplasm with bluish granules scattered throughout the egg. These granules were somewhat diffusely stained and were seen with difficulty. They became concentrated toward the center of the egg about 25 minutes after the end of irradiation. None of these eggs divided. Many cytolized.

Inseminated *Lytechinus* eggs were transferred to 1:800,000 solution of Janus green in sea water 5 minutes after insemination and kept in diffuse light. In these eggs, very faintly stained granules were seen scattered throughout the cytosome. These eggs began cleavage simultaneously with those of the sea-water control 37 minutes after insemination. They continued their cleavage normally to the 12-cell stage. Later cleavage was abnormal, as was that in the sea-water control. Similarly, eggs from the inseminated stock were transferred to 1:800,000 solution of Janus green in sea water 5 minutes after insemination and irradiated and stirred for 20 minutes. The cytoplasm in these eggs became bluish. The granules were rather poorly stained. The eggs began to enter the 2-cell stage 40 minutes after insemination, this being about 3 minutes slower than in the control and in the culture in diffuse light. They continued this slightly slower cleavage for about 3 hours, after which they became abnormal as described above for the control and for the culture in diffuse light.

It will be noted that the duration of irradiation for all the cultures thus far described was 20 minutes. It may be noted further that following irradiation of this length there was cleavage only in the 1:800,000 solution of Janus green in sea water.

A series of experiments was then performed in which the length of irradiation was reduced to 10 minutes. Inseminated eggs, transferred to a 1:400,000 solution of Janus green in sea water 6 minutes after insemination and kept in diffuse light at a temperature of 29° C., retained a fine spherical form. These eggs cleaved regularly to 2-cell and 4-cell stages. They did not develop uniformly beyond this stage; 6 hours later the culture showed only 2-, 4-, and 8-cell stages together with a few abnormal later stages. Inseminated eggs, transferred to 1:400,000 solution of Janus green in sea water 6 minutes after insemination, and irradiated and stirred for 10 minutes with the temperature rising to 32° C., showed blue granules slightly below the surface. The eggs then became irregular in outline. Later all the eggs became indented. Within 5 minutes, or about 15 minutes after the end of irradiation, some of the eggs began to smooth out, while some were cytolizing. None of these eggs had divided an hour after insemination. The nuclei in most eggs showed rounded bodies. During the next 6 hours some of the eggs cleaved as far as the 4-cell stage, but many remained undivided and fully 50 per cent cytolized. In the experiment just described, therefore, there was no normal cleavage beyond the 8-cell stage in the culture in diffuse light, and in the irradiated culture only a few eggs reached the 4-cell stage and half the eggs cytolized. It seemed clear that the 1:400,000 solution was too strong to permit normal cleavage.

Work with 1:500,000 solution of Janus green in sea water was then undertaken. Inseminated eggs were transferred to 1:500,000 Janus green 5 minutes after insemination and kept in diffuse light. These eggs during the first 20 minutes in the dye remained spherical and then became slightly irregular. They formed good 2-cell stages, cleavage beginning 37 minutes after insemination.

The eggs were followed through a regular cleavage as far as the 12-cell stage. Inseminated eggs of the same lot were transferred to 1:500,000 solution of Janus green in sea water 5 minutes after insemination, and irradiated and stirred for 10 minutes. Most of these eggs became slightly irregular in form within 2 minutes after the end of irradiation. Within the next 20 minutes the contours of some of the eggs had become smooth and these eggs began to enter the 2-cell stage. Following this many more of the irregular eggs smoothed out, although many still remained rough, and finally nearly all became smooth and entered the 2-cell stage. A few undivided eggs cytolized. These eggs were followed through a regular cleavage to the 8-cell stage.

Inseminated *Lytechinus* eggs were transferred to 1:600,000 solution of Janus green in sea water 5 minutes after insemination and kept in diffuse light at a temperature of 28.8° C. Granules in these eggs were staining blue within 7 minutes. The eggs were somewhat slower in their cleavage than those of the sea-water control. They cleaved regularly up to the 32-cell stage and later formed somewhat abnormal swimming blastulae. Inseminated eggs transferred to 1:600,000 solution of Janus green in sea water 5 minutes after insemination and irradiated and stirred for 10 minutes, the temperature rising to 31.4° C., showed granules bluer than those in the diffuse-light culture. About 10 per cent of these eggs were in the 2-cell stage 50 minutes after insemination; 2 hours later the culture showed 2-, 4-, 8-, 12-, and 16-cell stages together with a few undivided eggs. The culture 6½ hours after insemination contained abnormal swimming blastulae and irregular cleavage stages, and showed some secondary cytolysis.

A series of experiments was then undertaken with 1:500,000 solution of Janus green. Inseminated eggs were transferred to solution of Janus green 5 minutes after insemination and kept in diffuse light at a temperature of 29.2° C. These eggs cleaved to the 16-cell stage. Eggs of the same insemination were transferred to 1:500,000 Janus green 5 minutes after insemination, and irradiated and stirred for 12 minutes, during which the culture reached the temperature of 31.2° C. These eggs were slightly irregular at the end of irradiation. The irregularity increased until it involved most of the eggs, but there was no cytolysis. Cleavage was somewhat slower than in the control but proceeded regularly during the 2¼ hours that it was followed, when the culture contained good 1-, 2-, 4-, 8-, and 12-cell stages as well as a few irregular undivided eggs.

The irregularity in recovery of eggs in the nonirradiated cultures, when compared with previous results, suggested that the Janus green stock solution had undergone some deterioration. A new solution was therefore made. The stock solution used was 12 days old, but it had not been used since the end of the experiments involving 20-minute irradiation a week previously. It should be particularly noted, as has been stated, that the irregularity of the results was in recovery of eggs in the nonirradiated cultures. The question of duration of irradiation does not enter here.

A new set of experiments with freshly prepared stock solution was performed with various durations of irradiation and with controlled temperature. Inseminated *Lytechinus* eggs were transferred to 1:500,000 solution of Janus green in sea water 5 minutes after insemination at a temperature of 28.6° C. and kept in diffuse light. The granules in these eggs stained slightly. During the first 25 minutes after being placed in the solution of Janus green the eggs became slightly rough. They gradually became smooth and about half of them had entered the 2-cell stage 44 minutes after insemination. Those in the control developed in the same tempo. During the next 5 minutes nearly all had entered the 2-cell stage. Cleavage in these eggs proceeded regularly and 7½ hours after insemination most were nearly normal swimming blastulae like those in the sea-water control.

The remaining experiments of this set involve irradiations of different lengths and increased temperature. Inseminated eggs were transferred to 1:500,000 solution of Janus green in sea water 5 minutes after insemination, and irradiated and stirred for 10 minutes, the temperature rising to 31.8° C. These eggs became slightly rough during irradiation. This roughness increased for a few moments and then during the first 20 minutes after the end of irradiation gradually disappeared. About half of these eggs were in the 2-cell stage 45 minutes after insemination, although 5 minutes later many were still in anaphase. The eggs continued their cleavage normally in the same tempo as those of the control and of the diffuse-light culture. The cultures showed many normal swimming blastulae, more normal than those in the succeeding culture, 7½ hours after insemination.

Inseminated eggs of the same lot were transferred to 1:500,000 solution of Janus green in sea water 5 minutes after insemination, and irradiated and stirred for 15 minutes, the temperature rising to 32.6° C. Eggs in this culture were rougher than those in the preceding culture at the end of irradiation. In these again, the roughness gradually disappeared, although 20 minutes after the end of irradiation 50 per cent were still rough and 30 minutes after the end of irradiation at least 25 per cent were still rough. The eggs in this culture began to divide 45 minutes after insemination and proceeded slowly, lagging somewhat behind those of the sea-water control and of the culture just described. One and a half hours after insemination the lag shown by this culture was appreciable. The culture contained very irregular swimming blastulae and many cytolized eggs 7½ hours after insemination.

Eggs transferred to 1:500,000 solution of Janus green in sea water 5 minutes after insemination, and irradiated and stirred for 20 minutes, at the end of which the temperature had risen to 34.2° C., were rougher at the end of irradiation than any noted above. Most of the eggs in these cultures 55 minutes after insemination or 30 minutes after the end of irradiation were rough and undivided; some were smooth and undivided; a few had divided irregularly, and still fewer had cytolized. During the next hour cytolysis increased and there was an increased number of irregular divisions until there were numerous irregular 4-cell stages. Most of these eggs, however, remained rough and undivided. Seven and one half hours after insemination, the culture showed highly irregular 1-, 2-, 3-, 4-, 7-, and 8-cell stages and many cytolized eggs. There was no development to swimming blastulae. The temperature noted for this last culture, 34.2° C., was too high for normal development. It is impossible to say how much of the effect was due to irradiation in solution of Janus green and how much to the increased temperature. As a check on this, another experiment was performed in which inseminated eggs of the same lot were transferred to sea water, and irradiated and stirred for 20 minutes, during which the temperature rose to 33.3° C. This experiment was with sea water alone, no dye being used. In their initial reaction these eggs showed the same degree of roughness as those of the culture last described. Ten minutes later, however, these eggs were smooth, and 17 minutes after the end of irradiation (42 minutes after insemination) they were in or entering the 2-cell stage; some were still in anaphase. These eggs were distinctly in advance of those in the sea-water control. All the eggs of this culture were in the 2-cell stage 47 minutes after insemination, whereas those of the control had not reached an approximately similar stage 49 minutes after insemination. These eggs continued their cleavage, but many of the 8-cell stages were irregular. Cleavage continued, and many of the late cleavage stages with a large cleavage cavity looked nearly normal. Eight hours after insemination the culture contained many blastulae with mesenchyme. It is obvious that these eggs, irradiated in straight sea water, withstood the excessive temperature of 33.3° C. extremely well. The difference from the result of the experiment with 1:500,000 solution of the dye at 34.2° C. is striking.

On the same day similar experiments were performed with eggs from another female and a stronger solution of Janus green. Inseminated *Lytechinus* eggs were transferred to 1:400,000 solution of Janus green in sea water 5 minutes after insemination at 29.2° C., and kept in diffuse light. Granules in these eggs were stained bluish 5 minutes after the transfer. In about 30 minutes most of the eggs had become irregular in form. The irregularity was temporary, and after 15 minutes most of the eggs became smooth and showed anaphase figures. Development, however, was very slow. In 55 minutes after insemination, the culture showed some 2-cell stages, some smooth undivided eggs, and many irregular eggs; 20 minutes later, or an hour and a quarter after insemination, most of the eggs had divided or had become smooth and showed an amphiaster. Cleavage continued to be slow, and 3 hours after insemination the culture showed only 2-, 4-, and 8-cell stages.

Inseminated eggs transferred to 1:400,000 solution of Janus green in sea water 5 minutes after insemination, and irradiated and stirred for 10 minutes, during which the temperature in the culture rose to 32.2° C., were slightly irregular in outline at the close of irradiation, and the granules were bluer than those in the eggs in the diffuse-light culture. All the eggs in this culture had become irregular 15 minutes after the end of irradiation. This general irregularity continued until 28 minutes after the end of irradiation, when a few became smooth and showed an amphiaster. Fifty-five minutes after insemination, these eggs had nearly all become smooth and some had divided to normal 2-cell stages. Some of the eggs of this culture cytolized. When the eggs of the sea-water control were in the 4- and 8-cell stage, even the best eggs of this irradiated culture had reached only the 2-cell stage. The culture showed undivided eggs, 2-, 4-, and 8-cell stages, and some cytolized eggs, 4 hours after insemination.

Inseminated eggs were also transferred to 1:400,000 solution of Janus green in sea water 5 minutes after insemination, and irradiated and stirred for 15 minutes, during which the temperature rose to 33.4° C. These eggs became more irregular and the granules stained bluer than the eggs in the preceding culture. This irregularity continued until 25 minutes after the end of irradiation, when a few became smooth. At this time some eggs were blister cytolizing and most were very irregular in form. Many eggs were becoming smooth and developing amphiasters and a very few were dividing 1 hour and 7 minutes after insemination; 10 minutes later most of the eggs in the culture were irregular or cytolized; some became smooth and some irregular division to the 4-cell stage took place. The culture showed stages from undivided eggs to 8-cell stages and cytolized eggs 3¼ hours after insemination.

An experiment involving irradiation of eggs in sea water containing no dye was again performed. Inseminated *Lytechinus* eggs of the same stock were transferred to straight sea water 5 minutes after insemination, and irradiated and stirred for 15 minutes, during which the temperature rose to 32.4° C. These eggs all became irregular in form. Within 12 minutes after the end of irradiation they had become smooth and showed the presence of amphiasters. The eggs of this culture began to divide to 2 cells 38 minutes after insemination, the insemination-cleavage interval being the same as that of the sea-water control. All the eggs of this irradiated culture were in the smooth 2-cell stage 46 minutes after insemination. At this time some of the eggs of the sea-water control were in the late anaphase stage. The eggs of the sea-water control and the eggs of this irradiated culture in sea water were in good 4-cell or 8-cell stages 1 hour and 20 minutes after insemination. These two cultures were still uniform in appearance and had reached a late cleavage stage with a large blastocoele 3 hours and 20 minutes after insemination. It should be noted that many of the eggs in the sea-water control had become slightly irregular in form 26 minutes after insemination. This irregularity lasted only a few minutes, all the eggs of the control culture becoming smooth and beginning cleavage 38 minutes after insemination. It is clear that the reaction of these eggs in sea water to irradiation in sunlight at the temperatures noted above, 33.3° C. and 32.4° C., was reversible, and that the eggs recovered from their injury and proceeded to normal cleavage. It is also evident that in the solution of dye the reaction was not reversible.

The remaining experiments were all performed with 1:500,000 solutions of Janus green in sea water. In the first of these experiments inseminated *Lytechinus* eggs were transferred to 1:500,000 solution of Janus green in sea water 5 minutes after insemination and kept in diffuse light at a temperature of 28.2° C. These eggs were stained light green with more deeply stained granules within 5 minutes. During the next 25 minutes the contour of most of the eggs remained smooth and the cytoplasm stained uniformly. During the succeeding 10 minutes the contour of many of the eggs in the culture became irregular, while that of others remained smooth. The eggs of the sea-water control were entering the

2-cell stage 55 minutes after insemination; in the dye culture in diffuse light there were rare 2-cell stages, but many eggs showed anaphase figures. The dye culture in diffuse light 1¾ hours after insemination contained undivided eggs and 2-cell and 4-cell stages, and the sea-water control contained 8-, 12-, and 16-cell stages. When the cultures were compared 4 hours and 20 minutes after insemination, the dye culture in diffuse light contained 4-cell and 8-cell stages as well as irregular eggs, and the control in sea water contained normal hollow spheres.

Inseminated eggs transferred, 5 minutes after insemination, to 1:500,000 solution of Janus green in sea water, of a temperature of 28.2° C., were irradiated and stirred for 10 minutes. This culture was cooled during irradiation and at the end of irradiation its temperature was 27.6° C. These eggs were more deeply stained than those of the culture in diffuse light, and the granules were sharply stained. The contour of these eggs became irregular 12 minutes after the end of irradiation. The eggs of all the irradiated cultures, this one and the two others that will be described, showed a clear periphery, a more deeply stained core, and a lighter spot, larger than the nucleus, in the center. The contour of most of the eggs had become smooth while the contour of others remained irregular 25 minutes after the end of irradiation. The plasma membrane on these eggs was not, or was only slightly, lifted. Most of the eggs were undivided and contained an intact nucleus 85 minutes after insemination. Only one of the eggs of this culture, 25 minutes later, was seen to be divided; most were inert with the plasma membrane slightly lifted, but some showed cytolysis. This culture 4 hours after insemination contained a few intact eggs; most of the eggs were cytolized and disintegrating.

Inseminated eggs transferred, 6 minutes after insemination, to 1:500,000 solution of Janus green in sea water, of a temperature of 28.2° C., were irradiated and stirred for 15 minutes. The temperature of this culture was lowered and at the end of irradiation stood at 27° C. The eggs of this culture were stained a deeper green than those of the preceding cultures. The contour of the eggs was irregular at the end of irradiation. There had been no cleavage 70 minutes after insemination. The plasma membrane was lifted in small waves from the surface of the egg. The nucleus remained intact. Lobular cytolysis appeared in the culture 1¾ hours after insemination. The culture showed some intact eggs, but most had cytolized and were disintegrating 4 hours after insemination.

For the final culture of this series, inseminated eggs were transferred 6 minutes after insemination to 1:500,000 solution of Janus green, of a temperature of 28.2° C. This culture was irradiated and stirred for 15 minutes, the temperature at the end of the procedure being 31.7° C. The eggs of this culture had an irregular contour 8 minutes after the end of irradiation. The deeply stained zone was more closely drawn toward the center, and the eggs were in general more deeply stained than in the preceding cultures. The contour of the eggs had become smooth 30 minutes after the end of irradiation. The plasma membrane on these eggs was lifted in small waves. The nucleus was intact 70 minutes after insemination. Lobular cytolysis began 60 minutes after the end of irradiation and increased gradually during the next half-hour until it approached 50 per cent. All the eggs were cytolized and disintegrated 4½ hours after insemination.

The final experiments to be reported were done with uninseminated *Lytechinus* eggs in 1:500,000 solution of Janus green in sea water. In this experiment, because of the temporary difficulty of obtaining them, mixed eggs of three females were used. Uninseminated *Lytechinus* eggs were transferred to 1:500,000 solution of Janus green in sea water and kept in diffuse light at a temperature of 28.7° C. for 11 minutes, when they were inseminated. Their fertilization membranes were poorly separated 3 minutes after insemination, whereas those of the sea-water control were 100 per cent well separated. The fertilization membranes were distinctly separated 15 minutes later. The eggs of the control began cleavage 38 minutes after insemination; those of the diffuse-light culture showed some amphiasters, but the nucleus was intact in most of the eggs 43 minutes after insemination. About 10 per cent of the eggs in these dye cultures in diffuse light were in the 2-cell stage 59 minutes after insemination. More than 50 per cent had reached a regular 2-cell stage 15 minutes later. The eggs of the control had developed to hollow spheres 4 hours after insemination, and those of the diffuse-light culture were in a late cleavage stage with a very small cleavage cavity.

Uninseminated eggs were transferred to 1:500,000 solution of Janus green in sea water and irradiated and stirred for 10 minutes. During irradiation the culture was cooled and held at 28.9° C. The eggs were inseminated at the end of irradiation. They were stained blue and had poorly separated membranes 3 minutes after insemination. During the next 15 minutes the surface of most of these eggs became wrinkled, a wrinkling that involved the plasma membrane as well as the fertilization membrane. About 2 per cent of these eggs had reached the 2-cell stage 67 minutes after insemination. Cleavage did not proceed normally; about half the eggs were blistered and the remainder had formed irregular clumps 4 hours after insemination.

Uninseminated *Lytechinus* eggs were irradiated and stirred in 1:500,000 solution of Janus green in sea water for 20 minutes. The culture during this time was cooled and at the end of irradiation its temperature was 28.8° C. The eggs were inseminated at the end of irradiation. Examination under high magnification (4D) showed poorly separated fertilization membranes; the eggs were very blue, and there was a region of deeply stained material between the cortex and the center of the egg. During the first 14 minutes following insemination, the surface of the eggs remained smooth and the color in the zone of deeply stained material diffused into the surrounding substance of the egg. The surface of the eggs became rough during the next 10 minutes, although there was no blistering. Within an hour after insemination, however, blister cytolysis was present to the extent of 10 per cent. No regular cleavage occurred in this culture, and 4 hours after insemination all the eggs were in the form of irregular blistered clumps.

For the final culture, uninseminated eggs were transferred to 1:500,000 solution of Janus green in sea water. These were irradiated and stirred for 20 minutes. The temperature of the culture was held as closely as possible to 32° C. It stood at 31.8° C. at the end of irradiation. The eggs were then inseminated, and 2 minutes later showed a very close, poorly separated fertilization membrane. About 1 per cent of the eggs, 15 minutes after insemination, were blistered. These eggs, like those of the preceding culture, showed a zone of deeply stained material between the cortex and the center of the egg. This color diffused out into the cytoplasm. The eggs had become more deeply stained during the first 25 minutes after insemination, but blistering was not much increased and the surface of the eggs was not greatly roughened. During the next 15 minutes, however, blister cytolysis had increased to about 10 per cent, and an hour after insemination it had reached a height of 40 per cent and disintegration had begun. All the eggs of this culture had blistered and formed irregular clumps within 4 hours after insemination. The increase of 3° in temperature over that of the preceding experiment seems to have had no influence on the final results.

Results of the Study of Sections of Eggs from Janus Green B (Diazine Green) Cultures

In nonirradiated 1:400,000 solution of Janus green in sea water, about 40 per cent of the eggs had anaphase figures which were slightly irregular, and there was some lagging of chromosomes; in 1:500,000 solution, the chromosomes in anaphase plates were slightly attenuated; in 1:600,000 nonirradiated solution, 20 per cent were lagging and 80 per cent were normal chromosomes.

In 1:400,000 solution of Janus green in sea water irradiated 10 minutes, the chromosomes were irregular and compact (fig. 6). In 1:500,000 irradiated solution they were attenuated and 90 per cent showed slight lagging. This peculiarity

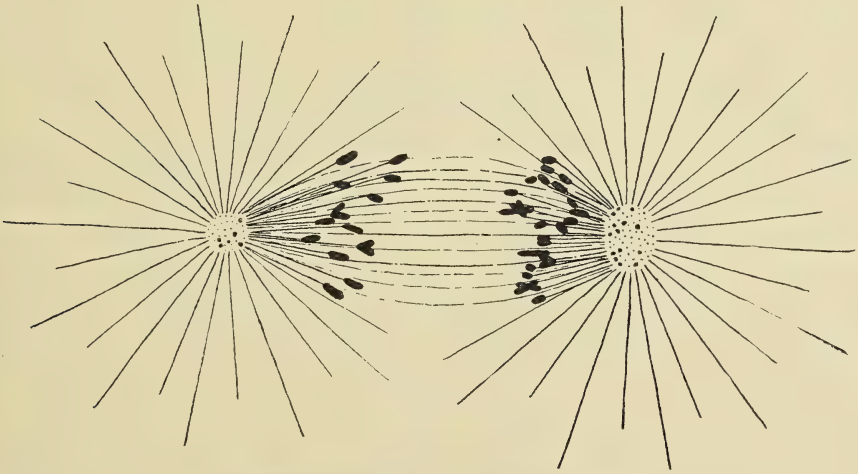


FIG. 6. Janus green B, 7/26/36, 5:05 P.M.

persisted into the second division. In 1:600,000 irradiated solution of Janus green the chromosomes showed irregularity and lagging.

In 1:400,000 solution in which the temperature during a 15-minute irradiation rose to 33.4° C., the anaphase plates were much more irregular than following 10-minute irradiation at 31.8° C. The chromosomes became spherical in form and showed an irregular grouping (fig. 7).

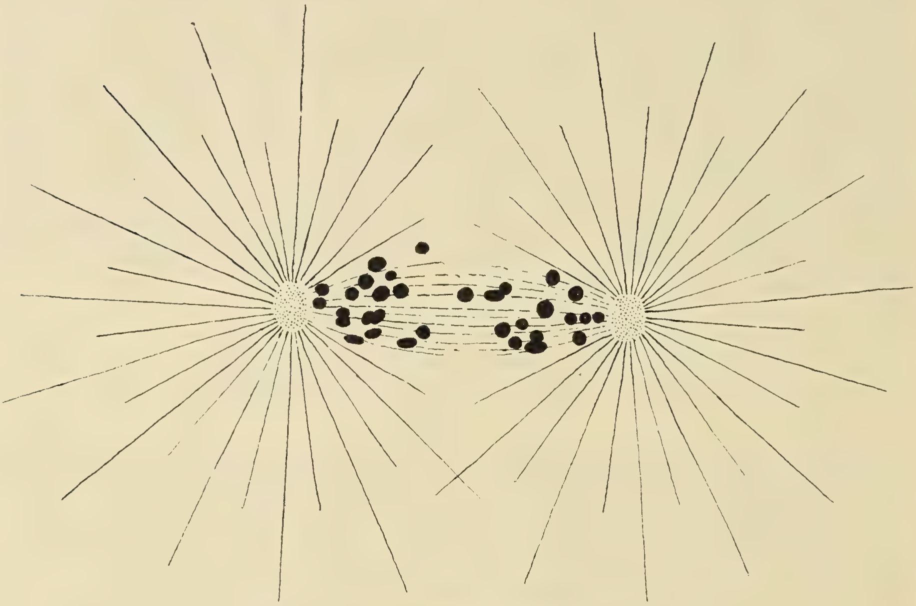


Fig. 7. Janus green B, 7/26/36, 5:17 p.m.

Congo Red

The dye used was Coleman and Bell's Congo red 4B. It was first used in 1:150,000 solution in sea water. The first experiments performed were for the purpose of determining the possible difference in reaction of inseminated and uninseminated eggs to solutions of Congo red in sea water.

In the first series of experiments, uninseminated *Lytechinus* eggs were transferred to 1:150,000 Congo red at a temperature of 29.6° C. and inseminated 11 minutes later in this solution. Eggs in the sea-water control formed excellent fertilization membranes, completed their first cleavage 39 minutes after insemination, and 2 hours later had reached fine late cleavage stages. Eggs in the solution of Congo red formed no fertilization membranes. The nuclei of these eggs remained intact. The eggs remained unchanged 3 hours later, showing no development. Uninseminated eggs were transferred to solution of Congo red, were irradiated and stirred for 10 minutes, the temperature being held at 29.6° C., and were inseminated 1 minute after the close of irradiation. These eggs formed no fertilization membranes and during the next 3 hours remained unchanged and showed no development. Uninseminated eggs were transferred to solution of Congo red in sea water, were irradiated and stirred for 20 minutes, the temperature being held at 30° C., and were inseminated 2 minutes after the end of irradiation. These eggs showed no fertilization membranes and remained unstained. Many of the eggs became vacuolated, but during the next 2 hours showed no development. Uninseminated eggs were transferred to solution of Congo red, were irradiated and stirred for 20 minutes, the temperature being kept at 32° C., and were inseminated 2 minutes after the close of irradiation. They remained unstained and formed no fertilization membranes. Some of these eggs likewise became vacuolated. During the next 2 hours vacuolization increased somewhat, but the eggs remained unchanged.

The reaction of the inseminated *Lytechinus* eggs when transferred to 1:150,000 solution of Congo red in sea water was decidedly different. The eggs in the sea-water control for these experiments began to cleave 36 minutes after insemination. The inseminated eggs that were transferred to 1:150,000 Congo red and held at a temperature of 30.6° C. showed no effect of the dye. The perivitelline liquid remained unstained. The eggs in this culture, during early cleavage, kept pace with those of the control. Inseminated eggs that were transferred to 1:150,000 Congo red, irradiated and stirred for 10 minutes, and kept at a temperature of 30.6° C. remained smooth and spherical. The eggs were not stained, but the perivitelline liquid became pink. These eggs divided uniformly and regularly, keeping pace with the control. Inseminated eggs that were transferred to 1:150,000 Congo red, irradiated and stirred for 20 minutes, and held at a temperature of 30.5° C. remained smooth and spherical. These eggs remained unstained but showed slight pink coloration in the liquid between the fertilization membrane and the egg. These eggs likewise divided regularly like those in the control. Inseminated eggs that were transferred to 1:150,000 solution of Congo red, irradiated and stirred for 20 minutes, and held at a temperature of 32° C. (actually 31.9° C.) behaved precisely as did the eggs in the other cultures of this series. They remained smooth and spherical, showed pink coloration in the perivitelline liquid, and showed the same rhythm of cleavage as the eggs in the sea-water control.

The difference in behavior of the eggs in these two series of cultures is striking. This difference is precisely that which we have learned to associate with unfertilized and fertilized *Lytechinus* eggs in solutions of acid and basic dyes. When uninseminated eggs were placed in a solution of Congo red, an acid dye, the dye entered, forming vacuoles. When inseminated eggs were placed in similar solutions of Congo red, apparently only a very slight amount of the dye made its way through the fertilization membrane, and the concentration of dye in the perivitelline liquid did not become great enough to be taken into the egg in the form of vacuoles. These facts will be considered in greater detail in the presentation of the work done with eosin Y and fluorescein.

The remainder of the work with Congo red 4B was done on uninseminated eggs with solutions ranging in concentration from 1:150,000 to 1:600,000.

The experiments of the next series were followed only until it became evident that there would be no development or change in the experimental material.

Eggs of the sea-water control upon insemination formed excellent fertilization membranes and cleaved regularly and normally. Uninseminated eggs transferred to 1:150,000 Congo red at a temperature of 29.3° C. were inseminated in this solution 11 minutes after the transfer. They formed no fertilization membranes, the nucleus remained intact, and the eggs underwent no development. Uninseminated eggs transferred to 1:150,000 Congo red, and irradiated and stirred for 10 minutes with the temperature held at 29.3° C., were inseminated 1 minute after the end of irradiation. These eggs formed no fertilization membranes and underwent no cleavage or development during the next 3½ hours. Uninseminated eggs transferred to 1:150,000 Congo red, irradiated and stirred for 20 minutes with the temperature kept at 29.3° C., and inseminated 1 minute after the end of irradiation formed no fertilization membranes and underwent no development or change, eggs and nuclei remaining intact. Uninseminated eggs were transferred to solution of Congo red, irradiated and stirred for 20 minutes with the temperature kept at 32° C. (actually 31.9° C.), and inseminated 1 minute after the end of irradiation. These eggs likewise showed no fertilization membranes and no change or development during the 3 hours following insemination.

In the next experiment a concentration of 1:300,000 Congo red 4B was used. The eggs in the sea-water control formed excellent fertilization membranes and proceeded through regular cleavage. Uninseminated eggs transferred to 1:300,000 Congo red and inseminated in this solution 10 minutes after their transfer showed no fertilization membranes. These eggs underwent no development. Uninseminated eggs transferred to 1:300,000 Congo red, irradiated and stirred for 10 minutes at a temperature of 30.9° C., and inseminated at the end of irradiation showed no fertilization membranes. These eggs showed during the next 2 hours very rare irregular cleavage. Uninseminated eggs that were transferred to solution of Congo red 4B, irradiated and stirred for 20 minutes at 30.8° C., and inseminated at the end of irradiation showed no fertilization membranes. During the next 2 hours these eggs showed rare irregular cleavage. Uninseminated eggs were transferred to 1:300,000 Congo red, irradiated and stirred for 20 minutes at a temperature of 32° C. (actually 32.1° C.), and inseminated at the close of irradiation. These eggs showed no fertilization membranes, but they showed rare irregular cleavage during the next 2 hours.

The final series of experiments was with 1:600,000 Congo red. The eggs in the sea-water control for these experiments showed rather poor fertilization membranes, imperfectly detached. Cleavage in the control was regular and resulted in the formation of rather poor late cleavage stages. Uninseminated eggs were transferred to 1:600,000 solution of Congo red at 29.4° C. and at the end of 10 minutes inseminated in this solution. These eggs—and the same is true of the eggs in the remaining experiments in the series—showed no fertilization membranes and showed rare but imperfect fertilization reactions. Many eggs appeared like those in the sea-water control. Some of the eggs were constricting for the 2-cell stage 41 minutes after insemination. They formed many rather poor late cleavage stages. Uninseminated eggs were transferred to

1:600,000 Congo red, irradiated and stirred for 20 minutes at a temperature of 29.6° C., and inseminated 1 minute after the end of irradiation. These eggs formed no fertilization membranes, but showed some rare fertilization reactions. Some eggs in this culture developed as far as late cleavage stages, but fewer developed than in the culture just described. Uninseminated eggs were transferred to 1:600,000 Congo red, irradiated and stirred for 20 minutes at a temperature kept at 32° C. (actually 31.6° C.), and inseminated 1 minute after the end of irradiation. These eggs also showed no fertilization membranes and rare but imperfect fertilization reactions. During the next 4 hours the eggs of this series showed some rare blistering and some cleavage to poor late cleavage stages. This culture showed the fewest cleavage stages of any of these cultures.

Alizarin Red S

Two concentrations of alizarin red S, Coleman and Bell, were used, neither showing any photodynamic effect. The first concentration used was 1:50,000. *Lytechinus* eggs were placed in this solution 6 minutes after insemination. The eggs in the nonirradiated culture showed no evidence of stain. They began to cleave 42 minutes after insemination, being slightly slower than the eggs in the sea-water control. Cleavage proceeded regularly, the eggs giving rise to normal young plutei. Similarly inseminated eggs placed in this solution and irradiated and stirred for 10 minutes showed no stain in the egg although a pink color was evident in the perivitelline space. These eggs also cleaved regularly, the insemination-cleavage interval being the same as in the nonirradiated eggs; they gave rise to normal young plutei. Because of the ineffectiveness of the 1:50,000 solution, experiments with 1:25,000 solutions were undertaken. *Lytechinus* eggs 5 minutes after insemination were placed in diffuse-light cultures. These eggs remained unstained, although the perivitelline liquid was stained slightly pink. The eggs began to enter the 2-cell stage 36 minutes after insemination. Cleavage and development followed regularly, the eggs giving rise to normal young plutei. In inseminated eggs placed in 1:25,000 solution and irradiated and stirred for 10 minutes, a pink color was also present in the perivitelline space and the nucleus was stained slightly pink. The eggs in this culture also began to cleave 36 minutes after insemination, and, as in the nonirradiated culture, proceeded regularly through the early stages of development and gave rise to normal young plutei. In both these cultures there was considerable precipitation of the dye, greater in the irradiated than in the nonirradiated cultures.

It is thus evident that in both of the concentrations used, the dye not only was nontoxic in the nonirradiated cultures, but showed no photodynamic effect following irradiation.

Thionin

Grübler's thionin was used throughout this work, in concentrations varying from 1:50,000 to 1:200,000. The solutions were made by dissolving the dye directly in sea water.

Lytechinus eggs were transferred 5 minutes after insemination to 1:50,000 solution of thionin in sea water and kept in diffuse light. They cleaved regularly, the tempo of their cleavage being the same as that of those in the sea-water control. Cultures were also made by transferring eggs 5 or 6 minutes after insemination to 1:50,000 solution of thionin in sea water and irradiating and stirring these for 10 minutes. In all these eggs there was much cytolysis and there were some abortive first divisions. Rounded bodies appeared in the nucleus. Usually the eggs became exceedingly irregular in form, with projecting buds. The nuclei disappeared, but no normal cleavage was ever observed.

Eggs transferred to 1:100,000 solution of thionin in sea water 5 minutes after insemination and kept in diffuse light cleaved normally in the same tempo as in the sea-water control and gave rise to gastrulae, all of which died before the following morning. Eggs of the same insemination transferred 5 minutes after insemination to 1:100,000 solution of thionin in sea water and irradiated and stirred for 10 minutes showed a large nucleus and some blister cytolysis 20 minutes after the end of irradiation. These eggs divided somewhat irregularly. The culture contained poor 2-cell stages and cytolized eggs when the eggs of the control and of the diffuse-light culture were in the 4-cell stage. Later these 2-cell stages smoothed up and divided to 4-cell and then to 8-cell stages. The culture contained, about 2 hours after insemination, 2-, 4-, and 8-cell stages and cytolized eggs. On the following morning all were dead amorphous masses.

The remaining experiments with thionin were done with unfertilized eggs. Uninseminated *Lytechinus* eggs were transferred to one-half saturated solution of thionin in sea water at a temperature of 29.1° C. and kept in diffuse light. They were inseminated 20 minutes later in this solution. Six minutes after being placed in the solution some eggs were exovate and all remained unstained. Fertilization membranes were well separated 3 minutes after insemination. All the eggs remained smooth. Constriction for the first division was in the same tempo as in the control, 38 minutes after insemination. The first irradiated culture was prepared by transferring uninseminated eggs to one-half saturated solution of thionin in sea water and irradiating and stirring them for 20 minutes with the temperature controlled. The eggs were inseminated at the end of irradiation; the temperature then stood at 29.2° C. The color of the irradiated solution faded during irradiation. The fertilization membranes were not separated, but remained closely applied to the slightly irregular surface of the eggs. None of the eggs blistered, but the surface of most became slightly pitted during the first 45 minutes after insemination. The nucleus of most eggs remained intact. Rare mitotic figures were visible 51 minutes after insemination. Most of the eggs in this culture had divided to poor 2-cell and 3-cell stages 1½ hours after insemination. Another culture was prepared by transferring uninseminated eggs to one-half saturated solution of thionin in sea water and irradiating and stirring for 20 minutes, the temperature not being controlled. These eggs were inseminated at the close of irradiation at a temperature of 35° C. This solution also faded during irradiation. The surface of the eggs was slightly irregular, with a tight fertilization membrane, 10 minutes after the end of irradiation.

Blistering began 17 minutes after insemination and during the next 2 minutes had increased to more than 10 per cent. About one-third of the eggs were blistered 5 minutes after its first appearance. The surface of most of the eggs was slightly pitted and about 35 per cent were blistered 40 minutes after insemination. The nucleus in all cases was intact. No mitotic figures appeared, and 1½ hours after insemination none of the eggs in the culture had divided.

The eggs in the diffuse-light culture in this half-saturated solution cleaved regularly; those irradiated for 20 minutes with the temperature controlled at normal became pitted and divided poorly; those irradiated for 20 minutes in this solution with the temperature allowed to rise to 35° C. failed to develop. The result of this experiment shows that irradiation at normal temperature produced a photodynamic effect, whereas irradiation in the solution of dye at 35° C. resulted in heat cytolysis which was superimposed on the photodynamic effect of the dye.

Finally, experiments were performed in which a saturated solution of thionin in sea water was used. For the diffuse-light culture, uninseminated eggs were transferred to a saturated solution of thionin in sea water at a temperature of 30.4° C. and inseminated in this solution 10 minutes after transfer. The fertilization membranes were not uniformly separated, but the eggs began to enter the 2-cell stage in tempo with the control 37 minutes after insemination. Their further cleavage was, however, slow; these eggs were still in the 4-cell stage when those of the control had reached the 8-cell stage, 83 minutes after insemination. For the first irradiated culture, uninseminated eggs were irradiated and stirred for 10 minutes. The temperature was controlled, and was at 30.7° C. when the eggs were inseminated at the end of irradiation. In these eggs the fertilization membrane remained tight. The color of the solution was not much changed. Although in the control and in the diffuse-light culture 2-cell stages began to appear 37 minutes after insemination, in this irradiated culture no divisions had appeared 1 hour after insemination. A few of the eggs had become vacuolated. About 15 per cent of the eggs had divided to the 2-cell stage 83 minutes after insemination. The final culture was prepared in the same way except that the uninseminated eggs were irradiated in the saturated solution of thionin in sea water for 20 minutes, the temperature being controlled during irradiation; it stood at 30.5° C. when the eggs were inseminated at the close of irradiation. The solution was slightly faded, the eggs were unstained, and no separated fertilization membrane was visible following insemination. Within the next 20 minutes 10 per cent of these eggs had become highly vacuolated, the vacuoles having refringent inclusions. The eggs remained in essentially the same condition during the next half-hour. Vacuolization had increased slightly and some cytolysis of the eggs was evident 70 minutes after insemination. The eggs failed to cleave. This experiment shows very clearly the result of prolonged irradiation in sunlight at normal temperature. The formation of vacuoles with inclusions in these eggs after 20 minutes irradiation was somewhat unexpected, this result with a strongly basic dye such as thionin being what one expects to find with an acid dye.

Results of the Study of Sections of Eggs from Thionin Cultures

In eggs irradiated in 1:50,000 solution of thionin in sea water there was enlargement of the nuclei and the occurrence of many nuclear inclusions. In many eggs the rims only of these rounded inclusions were deeply stained (fig. 8). In figure 9 there seems a possibility that material is being extruded into the cytoplasm. The nucleus is greatly enlarged and the size of the nucleoli considerably reduced. In figure 10, 2 nucleoli, a larger and a smaller, are in contact in the egg nucleus. In this figure there is also present a greatly enlarged male nucleus. In figure 11 a male nucleus and a female nucleus are shown in contact. The small protuberance is the male nucleus. In it the chromatin net has been reduced to chromosomes. In the eggs of this culture there were many protruding vacuoles with refringent inclusions. In the sections of these eggs there was much deeply stained flocculent material scattered throughout the cytoplasm. No division occurred.

THIAZINE RED

Thiazine red, Grübler, was used in this work. The following table presents the results of the investigation.

Summary of Results of Irradiation of Eggs in Solutions of Thiazine Red 1:20,000 and 1:40,000 for Various Lengths of Time

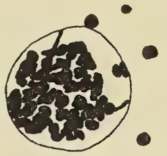
Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/10/39	(1)	28.6	1:40,000	0	Dark
	(2)	31.8	1:40,000	8	7969

Results. (1) 4 minutes after insemination no separated membranes; 26 minutes after insemination same as at 4 minutes after insemination; 43 minutes after insemination no division; 1 hour and 56 minutes after insemination unfertilized eggs, no change; on the following morning disintegrating eggs, some stained red; control, prism larvae and young plutei.

(2) 5 minutes after insemination no separated membranes; 26 minutes after insemination same as at 5 minutes after insemination; 2 hours and 1 minute after insemination unfertilized eggs, no change; on the following morning disintegration; some stained.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/11/39	(1)	29.6	1:20,000	0	Dark
	(2)	30.0	1:20,000	10	7305
	(3)	30.0	1:20,000	15	7305

[*Results on page 58*]



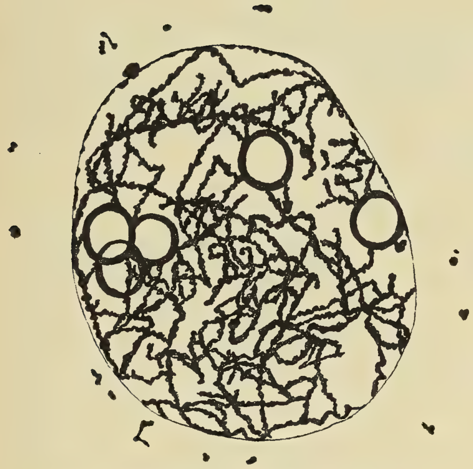


FIG. 8. Thionin, 7/23/36, 4:06 P.M.

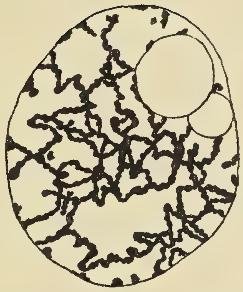


FIG. 10. Thionin, 7/23/36, 4:06 P.M.

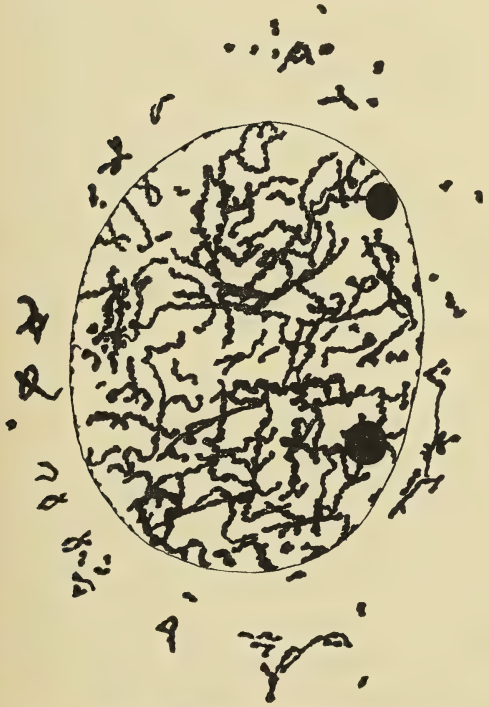


FIG. 9. Thionin, 7/23/36, 4:40 P.M.

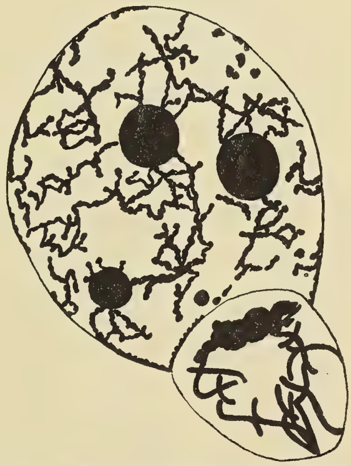


FIG. 11. Thionin, 7/17/37, 1:30 P.M.

Results. (1) 12 minutes after insemination no separated membranes; control excellent 2's 45 minutes after insemination; (1) 46 minutes after insemination no 2's; 3 hours after insemination no effect, no development; on the following morning all were cytolized, some stained, some not; control, prism larvae.

(2) 13 minutes after insemination no separated membranes; 47 minutes after insemination no 2's, no visible change; 3 hours and 5 minutes after insemination no effect evident, no development; on the following morning no development; the form of the eggs was retained.

(3) 8 minutes after insemination no separated membranes; 48 minutes after insemination no 2's, no visible change; 2 hours and 58 minutes after insemination no effect evident, no development; on the following morning no development; more disintegration than in (2).

METHYLENE BLUE

The preliminary work done with methylene blue in this research was with cultures in extremely dilute solutions which were not irradiated. In solutions of the concentrations 1:400,000, 1:200,000, and 1:100,000, the cleavage of inseminated eggs transferred to the solution 10 minutes after insemination was regular and in the tempo of the sea-water control. The contour of the eggs in all these solutions was smooth, the plasma membrane was good, and all the eggs showed blue granules in the cytoplasm. Within 5 minutes after being placed in the solution of dye the nucleolus appeared pink in contrast with the cytoplasm, although occasionally parts of the cytosome showed pink color. Unfertilized eggs were greenish, not blue. The distribution of the blue granules was uniform.

In a succeeding experiment unfertilized eggs were put in a 1:400,000 solution of methylene blue in sea water. After 10 minutes these eggs had become distinctly greenish. They were divided into two portions, one of which was kept as an unfertilized control in the solution of methylene blue in sea water. After remaining in this solution for another hour a portion was transferred to sea water and inseminated. The eggs formed excellent fertilization membranes. The granules in the eggs stained blue while the cytoplasm remained greenish. The second portion was fertilized in the solution of methylene blue 10 minutes after transfer to this solution. The fertilization reaction was poor, and the eggs were again inseminated 5 minutes later. The fertilization membrane was only slightly lifted, forming a wavy film. These eggs began to divide 39 minutes after insemination.

In the next experiments *Lytechinus* eggs were transferred 10 minutes after insemination to 1:100,000 solution of methylene blue in sea water and kept in darkness. These eggs were stained slightly bluish, and faint blue granules were evident 2 minutes after transfer to the solution. The eggs divided regularly and normally in the same tempo as in the control, and were followed to the 8-cell stage. Another portion was transferred 10 minutes after insemination to 1:100,000 solution of methylene blue in sea water and irradiated with sunlight for 10 minutes. At the close of irradiation these eggs had the same appearance as the eggs of the nonirradiated culture; they were stained slightly blue and showed blue granules. They cleaved with the same tempo and appearance as those in the control and the nonirradiated culture. They were followed only to the 8-cell stage.

The remaining work was done with stronger solutions. In the first of these experiments *Lytechinus* eggs were transferred to 1:20,000 solution of methylene blue in sea water 5 minutes after insemination at a temperature of 29.5° C. The eggs showed a sharp, regular outline and were stained blue 3 minutes after transfer to the solution. The blue granules were well stained and there were distinct radiations in the cytoplasm surrounding the nucleus 7 minutes after transfer to the solution. Cleavage began 35 minutes after insemination and continued regularly through late cleavage, all the eggs being stained very blue. Eggs of the same insemination were transferred 5 minutes after insemination to 1:20,000 solution of methylene blue in sea water and irradiated and stirred for 10 minutes. The temperature at the end of irradiation had reached 33.5° C. The eggs had stained very blue and showed good contours 3 minutes after the end of irradiation. Ten minutes later, the granules were more deeply stained and in sharp contrast with the cytoplasm; the eggs had become irregular. This irregularity continued and during the next 15 minutes involved all the eggs, some of which cytolized. A few 2-cell stages were found an hour after insemination, but most of the eggs had become highly irregular during this time, and no further development occurred.

The next experiments were done with methylene blue in the same concentration, with controlled temperature and with varying periods of irradiation. Inseminated eggs were transferred to 1:20,000 solution of methylene blue in sea water 6 minutes after insemination and kept in diffuse light at a temperature of 27.3° C. The eggs were stained diffusely 4 minutes after being placed in the solution, and 2 minutes later blue granules were visible. The eggs were very blue, with scattered blue granules, 27 minutes after being placed in the solution. They were entering the 2-cell stage 48 minutes after insemination. They continued normal cleavage in tempo with the control, but lagged somewhat behind the control in the formation of mesenchyme during the blastula stage. On the following day, 23 hours after insemination, the culture contained a few gastrulae, whereas the sea-water control contained plutei only. In diffuse light, therefore, methylene blue in 1:20,000 solution exerted a slight toxic effect on development.

Eggs of the same insemination were transferred 6 minutes after insemination to 1:20,000 solution of methylene blue in sea water and irradiated and stirred for 5 minutes. The controlled temperature at the end of irradiation was 28.3° C. These eggs, 3 minutes after the end of irradiation, were bluer than those in the diffuse-light culture, and were irregular in outline. They were entering the 2-cell stage 48 minutes after insemination and continued regular cleavage in tempo with the sea-water control. None of these eggs had developed to swimming blastulae and many were disintegrating 10 hours after insemination. On the following morning all had disintegrated.

Eggs of the same insemination, transferred to 1:20,000 solution of methylene blue in sea water 6 minutes after insemination, were irradiated and stirred for 10 minutes. At the end of irradiation the controlled temperature was 28.2° C. These eggs, 2 minutes after the end of irradiation, were bluer than those of the 5-minute irradiation. They had become very blue and were irregular in outline 20 minutes later. Most of the eggs divided rather regularly during the first two cleavages, although the irregularity in form continued. Following the second cleavage, however, they lagged behind those in the sea-water control and in the diffuse-light culture. The lagging became increasingly evident during the later cleavage. None of these eggs had reached the swimming-blastula stage and many were disintegrating 10 hours later, when the control showed formation of mesenchyme.

Inseminated eggs of the same lot, transferred to 1:20,000 solution of methylene blue in sea water 6 minutes after insemination, were irradiated and stirred for 15 minutes. The controlled temperature at the end of irradiation was 27.6° C. The eggs were stained very blue and contained large blue granules scattered throughout the cytosome but not aggregated around the nucleus. These granules were strikingly conspicuous because of their size. The outline of the eggs had become highly irregular 15 minutes after the end of irradiation. The eggs retained their irregularity of form during cleavage. The eggs of this irradiation lagged noticeably behind those in the other cultures; none had developed to swimming larvae and many were disintegrating 10 hours after insemination. On the following morning all had disintegrated. These results indicate that with irradiation 1:20,000 solution of methylene blue in sea water becomes toxic and its use results in disintegration of late cleavage stages. Experiments were therefore undertaken with a more dilute solution.

Lytechinus eggs were transferred, 5 minutes after insemination, to 1:40,000 solution of methylene blue in sea water and kept in diffuse light at a temperature of 28.2° C. These eggs, 4 minutes after transfer, were stained faintly blue with distinct blue granules. They were entering the 2-cell stage 38 minutes after insemination, being slightly slower than those of the sea-water control. The eggs in this culture were all in the 4-cell stage 1 hour and 25 minutes after insemination, when those in the sea-water control were in 4-cell and 8-cell stages. On the following morning this culture contained swimming gastrulae, and the control culture contained young plutei.

Eggs of the same insemination, transferred to 1:40,000 solution of methylene blue in sea water 5 minutes after insemination, were irradiated and stirred for 5 minutes. At the end of irradiation the controlled temperature was 29.8° C. The eggs were bluer than those of the diffuse-light culture, were spherical, and showed blue granules. These eggs began to enter the 2-cell stage 39 minutes after insemination. They continued cleavage regularly in the tempo of the eggs of the diffuse-light culture rather than in that of the sea-water control. On the following morning this culture contained swimming gastrulae only.

Eggs of the same insemination, transferred to 1:40,000 solution of methylene blue 5 minutes after insemination, were irradiated and stirred for 10 minutes. At the close of irradiation the controlled temperature was 29.8° C. These eggs were blue like those of the preceding culture, were irregular in form, and showed blue granules. A very few of these eggs were entering the 2-cell stage 40 minutes after insemination. They continued their cleavage slowly, being in the 2- and 4-cell stages when the sea-water control showed 4- and 8-cell stages, and the eggs of the 5-minute irradiation the 4-cell stage only. On the following morning this culture contained swimming gastrulae.

Finally, eggs of the same insemination, transferred to solution of methylene blue in sea water 5 minutes after insemination, were irradiated and stirred for 20 minutes. The controlled temperature at the end of irradiation was 27.6° C. All the eggs of this lot were regular in form, were stained blue, and showed blue granules. They showed no constriction 40 minutes after insemination. Seven minutes later, rare beginning 2-cell stages were in evidence, although many eggs were in anaphase. These eggs continued their cleavage in about the same tempo as those of the preceding culture, being somewhat slower in dividing than those of the sea-water control, of the diffuse-light culture, and of the culture irradiated for 5 minutes. On the following morning the eggs had reached the gastrula stage.

Results of the Study of Sections of Eggs from Methylene Blue Cultures

Inseminated *Lytechinus* eggs were transferred to 1:20,000 solution of methylene blue 5 minutes after insemination and fixed 30 minutes later. In prophase the centers were not large, but were clear, with astral rays extending to the surface of the egg. There was often a large nucleolus-like body on the central spindle. The chromosomes were not yet well differentiated; they looked like twisted threads. In metaphase the centers were not large. The central spindles were spindle-shaped, with astral rays extending to the surface of the egg. The chromosomes were sharply stained. There was often a deeply stained globule at one end of the central spindle. In mid-anaphase the chromosomes were rather diffusely stained, and difficult to distinguish from the spindle fibers. The centers were large and clear, with a few deeply stained granular central bodies. The astral rays extended to the surface of the egg. The central spindle was a straight band extending from center to center. In late anaphase the division figure had large, clear centers with deeply stained granular central bodies. The astral rays were numerous and many extended to the surface of the egg. The chromosomes were sharply stained, and some were lagging.

Inseminated *Lytechinus* eggs were transferred to 1:20,000 solution of methylene blue 5 minutes after insemination, irradiated for 10 minutes, and fixed 59 minutes after insemination. Development in these eggs was slower than in the nonirradiated eggs. The prophase nucleus had an aster with a small center at each pole. The chromatin was both granular and threadlike, and there was a large, deeply stained nucleolus-like body. In early anaphase the centers were medium in size and slightly opaque, with granular central bodies. The chromosomes were deeply stained, and their orientation was poor. There was a large nucleolus-like body at the edge of the spindle. Most of the later anaphase figures were irregular. The centers were opaque, with astral rays extending to the surface of the egg. There was a large nucleolus-like body at the edge of the central spindle. Irregularities in all the phases were common. Many of the eggs were cytolized. In these there were several fragments of the nucleus.

METHYLENE RED

Methylene red, Badische, was used in this work in 1:20,000 and 1:80,000 solutions. With both these concentrations there was some precipitation in sea water. As is shown in the following table, there was no development following insemination in either irradiated or nonirradiated dye solutions.

Summary of Results of Irradiation of Eggs in Solutions of Methylene Red 1:20,000 and 1:80,000 for Various Lengths of Time

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
6/24/39..........	(1)	27.3	1:20,000	0	Dark
	(2)	29.6	1:20,000	10	8234
	(3)	30.3	1:20,000	15	8234

Results. (1) No well separated fertilization membranes; no nuclear changes evident; no cleavage; control in 2's 50 minutes after fertilization, beginning 4's 65 minutes after fertilization.

(2) Post-inseminated; no well separated fertilization membranes; no 2's 55 minutes after insemination; no nuclear changes later; no cleavage; some eggs became pitted, many became very irregular.

(3) Post-inseminated; no well separated fertilization membranes, some with closely fitting fertilization membranes; no nuclear changes evident; no cleavage; some became pitted, many became very irregular; the methylene red solution formed a precipitate, when added to sea water, that settled very slowly, leaving the solution distinctly less colored than at first.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
6/25/39..........	(1)	28	1:80,000	0	Dark
	(2)	30.1	1:80,000	10	7703
	(3)	31.3	1:80,000	15	7703

Results. (1) No separated membranes; no division; no nuclear changes.

(2) Post-inseminated; no separated membranes; no division; no nuclear changes.

(3) Post-inseminated; 4 minutes after insemination no separated membranes; some pitting; no division; no nuclear changes.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
6/26/39..........	(1)	28.4	1:80,000	0	Dark
	(2)	31.7	1:80,000	10	8234–8766
	(3)	31.7	1:80,000	10	8234–8766

Results. (1) Post-inseminated; no separated membranes 3 minutes after insemination; no nuclear changes, no cleavage 5 minutes after controls were in 2's.

(2) Post-inseminated; no separated membranes 4 minutes after insemination; 5 minutes after controls were in 2's, no cleavage; no nuclear changes.

(3) Post-inseminated; no separated membranes 4 minutes after insemination; 5 minutes after controls were in 2's, no cleavage; no nuclear changes.

BRILLIANT CRESYL BLUE

The work with brilliant cresyl blue was done with concentrations of the dye (National Aniline Company, dye content 62 per cent) from 1:20,000 down to 1:160,000 in sea water. In staining with brilliant cresyl blue, the dye is taken up by the cytoplasm as well as by small spherical components in the cytoplasm. The cytoplasm became less blue as the granules took up color from it and became bluer. Experiments were performed for the purpose of determining whether the brilliant cresyl blue granules were the same components as those stained by solutions of neutral red. Since all the granules looked red in transmitted light, it was impossible to distinguish between brilliant cresyl blue and neutral red granules by direct observation.

After various experiments with reflected light and high dry objectives, it was decided to use stronger solutions of brilliant cresyl blue. It had been noted earlier that dilute solutions of brilliant cresyl blue were red in transmitted light, whereas more concentrated solutions were blue. It therefore seemed possible that the brilliant cresyl blue granules might be stained so strongly that they would no longer appear red in transmitted light.

In order to test this possibility, *Lytechinus* eggs were transferred to 1:20,000 solution of brilliant cresyl blue in sea water 15 minutes after insemination. Brilliant cresyl blue granules were faintly visible within 5 minutes. The intensity of staining increased gradually until the granules became dark blue and no longer showed red. The eggs were then examined under a D objective, 1:150,000 solution of neutral red being drawn under the cover slip. It was found that red centers appeared in the brilliant cresyl blue granules.

Eggs from the solution of brilliant cresyl blue were then placed in drops of 1:150,000 solution of neutral red and examined under the D objective. The same appearance of red centers in the blue granules was observed. No separate neutral red granules appeared.

It was therefore concluded that brilliant cresyl blue and neutral red stain the same bodies, the brilliant cresyl blue probably being absorbed on the surface of a vacuole-like structure, the neutral red staining the contents of the vacuole. The observations were thoroughly satisfactory, and were repeated under a 4-millimeter apochromat and under a 2-millimeter oil immersion apochromat.

Complementary experiments in which neutral red was used first, staining the neutral red granules, followed by brilliant cresyl blue, were performed. Ten minutes after insemination, *Lytechinus* eggs were placed in 1:150,000 solution of neutral red in sea water. Within 8 minutes neutral red granules appeared peripherally; these increased rapidly in number and size, lost their yellowish-red color, and became bright red. The eggs showed a peripheral zone free from granules within the next 5 minutes. When the eggs had reached the 2-cell stage they were transferred, some to 1:20,000 and some to 1:40,000 solution of brilliant cresyl blue in sea water. The cytoplasm of the eggs stained blue, showing a faint peripheral, diffusely stained ground substance; the initial reaction was followed by increased intensity of staining of the granules. They were of uniform size and stained diffuse light blue. Later the cytoplasm became bright blue and the red granules became dark. Finally the red disappeared completely, all the granules becoming blue. This experiment confirmed the conclusion that neutral red and brilliant cresyl blue stain the same granules or vacuoles.

The work with brilliant cresyl blue may be divided naturally into two parts: first, that done in 1935 and 1937 on inseminated eggs without measurement of the intensity of the sunlight; second, that done in 1938 on uninseminated eggs in which the intensity of the sunlight was measured.

Experiments in which the intensity of sunlight was not measured. In all this work the eggs were stirred constantly during their exposure to sunlight. The concentration of solution used varied from 1:20,000 to 1:80,000. The duration of irradiation was from 10 to 20 minutes. With concentrations of 1:20,000 and 10-minute irradiation, most of the eggs cytolized. With concentrations of 1:40,000 and 10-minute irradiation, irregular cleavage occurred and there was some blister cytolysis; with this concentration and irradiations of 15 minutes, cleavage was reduced, the eggs became lobose, and there was little cytolysis; with the same concentration and 20 minutes irradiation, cleavage was still further reduced and there was practically no cytolysis. With concentrations of 1:80,000 and 10 minutes irradiation, cleavage was irregular, some blister cytolysis occurred, and there was some abnormal development through gastrula and pluteus stages; with 15 minutes irradiation, cleavage was more irregular than following 10-minute irradiation, although blister cytolysis did not increase; with 20 minutes irradiation, irregularity of cleavage increased but blister cytolysis did not uniformly and invariably follow.

In this work the data at hand are as complete as could be desired, but the results are by no means so uniform as one might expect. The fact that different lots of eggs, treated by the same methods, show lack of uniformity in reaction is again emphasized. Since the temperature was controlled, irregularity in behavior cannot be attributed to effects of temperature.

Experiments in which the intensity of sunlight was measured. In the work done in 1938, measurements of the intensity of sunlight were made. The concentration of the solutions varied from 1:80,000 to 1:160,000. In this work some control of the intensity and of the wave length of the light reaching the experimental material was secured by the use of filters. The photodynamic effect of the dye is greater in the more dilute solutions than in the more concentrated solutions described above. The fact that there is great variation in the reaction of different lots of eggs is again emphasized by the behavior of one lot of eggs used on August 14, in which 25 per cent of the eggs in the nonirradiated dye control blistered although kept in darkness; the percentage of blistered eggs was not increased by irradiation. It may be noted further that there was blistering varying from 1 to 4 per cent in all the nonirradiated dye controls. Thus the effect of irradiation on the experimental cultures is not so great as it seems to be. If these facts were not taken into account, the results might be summarized as follows: With the highest intensities of sunlight used (8608 foot-candles), there was 20 per cent blistering; with an intensity of 7532 foot-candles, blistering was reduced to about 5 to 6 per cent; with reduction of intensity to 4300, blistering was reduced to 4 per cent. Two cases of irradiation at 2000 foot-candles, both through Corning filters no. 428, gave blistering exceeding in amount by 5 to 10 per cent that produced at 7532 foot-candles. The results of irradiation below 1000 foot-candles are not fundamentally different from those at 2000.

Attention should be drawn to the fact that the temperatures in cultures irradiated with sunlight of an intensity of 8600 foot-candles rose to 33° C. during the irradiation of 15 minutes. This excessive temperature is probably responsible for the high incidence of blisters.

As we have noted, moreover, there is initial blistering in the nonirradiated dye cultures that lessens the accuracy of the summary given above.

Attention must also be drawn to the fact that it was only at intensities of 8600, 7500, and 4300 foot-candles that the full range of the spectrum was used. The Corning filters no. 428 and no. 430 remove most of the red end of the spectrum and transmit the blue and green. The use of these filters therefore involved more than diminished intensity of irradiation. No essential difference was noted following irradiation through Corning no. 428 and no. 430 filters.

There is nothing in the results of the experimental work indicating an intensity threshold at which cytolysis may be expected.

Results of the Study of Sections of Eggs from Brilliant Cresyl Blue Cultures

In the control culture of these eggs the central spindle in both beginning 2- and beginning 4-cell stages was longer than in the experimental material of the same date. The centers were small, with astral rays reaching the surface of the egg; the chromosomes were not bent in the normal egg; the V-shaped chromosomes were prominent.

In the sections of the eggs that had been reared in nonirradiated 1:80,000 brilliant cresyl blue, the central spindle was short, there was a granular central body, and some astral rays reached the surface of the blastomeres. There were some lagging chromosomes which lay irregularly on the spindle. These eggs showed many irregular divisions, which were often associated with the abnormal orientation of one or many amphiasters. The result may be a row of 4 cells instead of a sphere of cells such as would be obtained by the second division of two hemispheres. A striking characteristic was the appearance of the centrosomes and asters; the centrosomes consisted often of a finely granular unstained sphere lying in a circular clear space that was crossed by spindle fibers. Outside this there was a dense layer of astral rays, some of which extended to the surface of the egg. This arrangement gave the centrosomes the appearance of a series of concentric zones. In other instances deeply stained irregular granular central bodies replaced the unstained finely granular central bodies. In these instances the clear space and the asters with astral rays reaching the surface persisted. The amphiasters for the second division may lie parallel to one another and parallel to the wall between the two blastomeres, or they may lie at an angle both to one another and to the central wall. As noted above, this irregular orientation of the amphiasters may be followed by exceedingly irregular cell division. In many instances the first division of the segmentation nucleus was not followed by division of the cytosome. In these instances the amphiasters of the second division were found lying side by side, each in one half of the undivided egg. The appearance of figures of this kind is similar to that of tetrasters, but no true tetrastral divisions were found. The amphiasters, although situated side by side, showed no chromosomal divisions between the two separate amphiasters. One triastral figure was noted in one half of an undivided egg in which the normal diaster was present in the other half. In the division of the chromosomes occasional lagging was evident, although this was not a characteristic feature in this lot of eggs. As implied above, the very common irregular division seems to be associated with the loss of orientation of the division figures.

In inseminated eggs irradiated for 10 minutes in 1:40,000 solution of brilliant cresyl blue, there was excellent cleavage. Few astral rays reached the surface of the egg, although some did. A perfect achromatic figure which was very short was characteristic of these eggs. The chromosomes were deeply stained and usually were very much bent. So far as chromosomes are concerned, no perfect mitotic figures were found. Two nucleoli often occurred on the central spindles. There was much lagging of chromosomes and irregularity in distribution.

In inseminated eggs transferred to 1:80,000 solution of brilliant cresyl blue and irradiated for 10 minutes, the surface of the eggs became covered with fine or large blebs. In some instances fine blister cytolysis covered the entire surface. In these eggs the mitotic figure was greatly reduced. This statement applies both to astral rays and to the central spindle. The centers were large, with deeply stained central granules. In many instances the spindle lay at right angles to the first cleavage plane. In other instances it lay at an oblique angle.

In inseminated eggs that had been irradiated 15 minutes in 1:80,000 solution of brilliant cresyl blue, the temperature never reaching 30° C. during irradiation, there was great irregularity in division. Many triasters and tetrasters were found, and the eggs were highly irregular in outline.

Finally, in inseminated eggs irradiated for 20 minutes in 1:80,000 solution of brilliant cresyl blue, with the temperature held below 30° C., there were many tripolar and tetrapolar spindles. The centers were small and the astral rays reduced. Many chromosomes were displaced. These figures often had a normal appearance, but the distribution of chromosomes was that shown in figures 12 and 13.

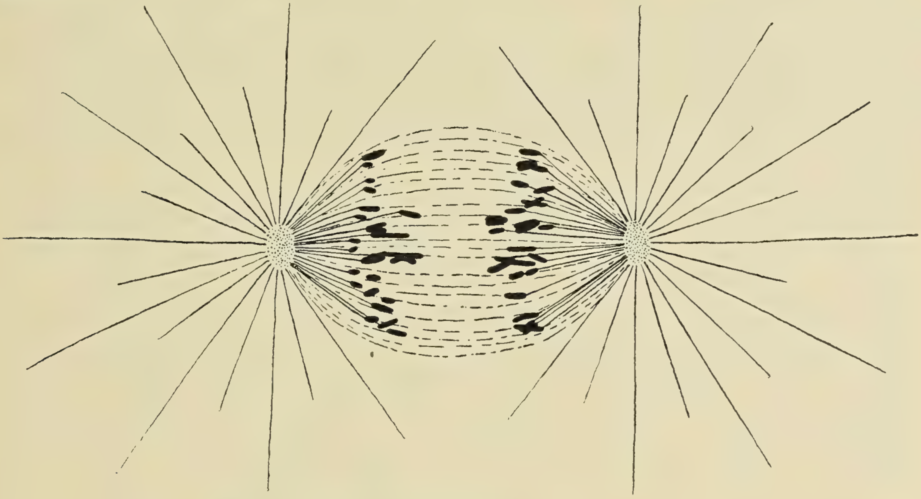


FIG. 12. Brilliant cresyl blue, 7/7/37, 3:27 P.M.

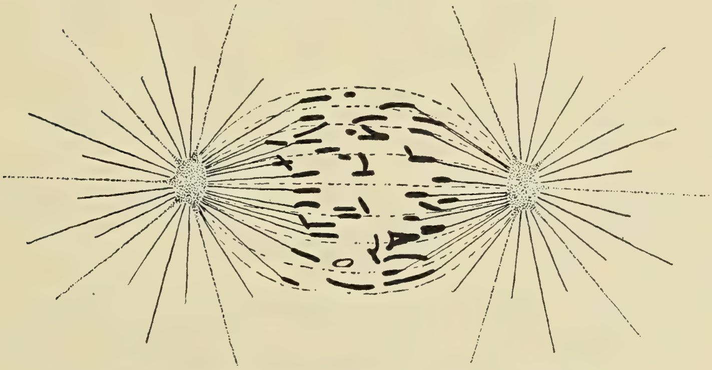


FIG. 13. Brilliant cresyl blue, 7/7/37, 3:27 P.M.

RESORCIN RED

Resorcin red, Badische, was used in this work. Development to the pluteus stage was normal in nonirradiated solutions, whereas in irradiated solutions plasmolysis and destruction of the surface of the egg occurred. The following table presents the results of the investigation.

Summary of Results of Irradiation of Eggs in Solutions of Resorcin Red 1:80,000

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
6/27/39..............	(1)	28.8	1:80,000	0	Dark
	(2)	29.1	1:80,000	10	8234
	(3)	29.2	1:80,000	15	8234

Results. (1) Post-inseminated; 19 minutes after insemination 10 per cent with well separated membranes; 64 minutes after insemination 10 per cent of eggs in 4's, remainder undivided; 1¾ hours after insemination 10 per cent 2's, 4's, 8's, 12's, 16's, remainder normal undivided eggs; 2 hours and 35 minutes after insemination 10 per cent in 64's, remainder undivided.

(2) Post-inseminated; 19 minutes after insemination less than 1 per cent separated membranes, less than 10 per cent pitted; 64 minutes after insemination none divided, less than 10 per cent vacuolated; 99 minutes after insemination eggs pink, 50 per cent pitted or with vacuoles containing inclusions; 2 hours and 35 minutes after insemination most of eggs with vacuoles have plasmolyzed.

(3) Post-inseminated; 14 minutes after insemination no separated membranes, 10 per cent pitting with destruction of surface of egg; 59 minutes after insemination eggs that had modified surface have disintegrated, remaining eggs smooth and unmodified, no nuclear changes, all eggs slightly pink; 99 minutes after insemination contents of many eggs plasmolyzed, many eggs have irregular protrusions; 1 hour and 38 minutes after insemination 50 per cent of eggs plasmolyzed, remainder look unchanged, eggs light pink.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
6/28/39..............	(1)	28	1:80,000	0	Dark
	(2)	30.4	1:80,000	10	8234
	(3)	30.4	1:80,000	15	8234

Results. (1) Post-inseminated; 2 minutes after insemination membranes well separated; 39 minutes after insemination, into 2's; 5½ hours after insemination blastulae rotating within fertilization membranes; on the following morning plutei with arms longer than plutei in the control.

(2) Post-inseminated; 2 minutes after insemination no fertilization membranes; 9 minutes after insemination more than 10 per cent with pits or vacuoles with inclusions; 35 minutes after insemination pitting less obvious; 4 hours and 16 minutes after insemination more than 10 per cent pitted, vacuolated, or disintegrated, most eggs pink; 5½ hours after insemination somewhat less than 10 per cent disintegrated, no normal development.

(3) Post-inseminated; 2 minutes after insemination no membranes, little pitting; 29 minutes after insemination more than 50 per cent plasmolyzing; 55 minutes after insemination above 60 per cent in plasmolysis; 70 minutes after insemination 90 per cent irregular and disintegrating, pink; 5½ hours after insemination above 90 per cent disintegrated, exceedingly irregular, clumps of egg fragments stained pink, many highly irregular masses with protrusions, these masses formed from single eggs; on the following morning all these eggs had disintegrated.

NILE BLUE A

In this work National Aniline and Chemical Company's Nile blue A, certified by the Commission on Standardization of Biological Stains, was used. The work with this dye was undertaken with more than usual interest because of the use of Nile blue sulphate as an indicator showing different color reactions to neutral fats, fatty acids, and mixtures of neutral fats and fatty acids.

The experiments in the attempt to use concentrations stronger than 1:500,000 have little significance, since upon the addition of solutions of Nile blue in distilled water to sea water to make solutions of higher concentration, the dye was precipitated in the form of fine needle-like crystals, leaving the sea water very faintly blue. The only report on concentrations of 1:100,000 to 1:500,000 that need be made is that inseminated eggs, placed in solutions of Nile blue A of these attempted concentrations, either in diffuse light or after irradiation in the solutions developed regularly and normally. Even in attempting to make a 1:500,000 solution in sea water in this manner there is some slight precipitation of the dye with consequent loss of the blue color.

Inseminated eggs, transferred to 1:600,000 solution of Nile blue A in sea water 6 minutes after insemination and kept in diffuse light, were stained bluish and showed numerous blue granules. These eggs were entering the 2-cell stage 40 minutes after insemination. They cleaved regularly in the tempo of the control and 23 hours later had developed to fine, large plutei. Eggs of the same insemination, transferred to 1:600,000 solution of Nile blue A in sea water 6 minutes after insemination and irradiated and stirred for 10 minutes, were stained very blue with numerous blue granules. The plasma also was stained, but more lightly than the granules. These eggs also cleaved in the same tempo as those of the control, although they were slightly slower in completing the 4-cell stage than those of the other cultures. The blastomeres were regular and of good form. In these eggs the blue granules were very sharply defined. The periphery was free from granules. These were clustered around the nucleus. Some granules had a clear center and a blue rim. On the following morning these eggs also had developed to fine, large plutei.

Eggs of the same insemination were transferred 6 minutes after insemination simultaneously with those of the above cultures to 1:1,200,000 solutions of Nile blue A in sea water. In the diffuse-light culture these eggs were stained slightly greenish. They developed in the same tempo as the control, giving rise to normal cleavage stages and on the following morning to excellent, normal, large plutei. Eggs of the same lot, transferred 6 minutes after insemination to 1:1,200,000 solution of Nile blue A in sea water and irradiated and stirred for 10 minutes, were stained light blue and showed light-blue granules. These eggs cleaved regularly and normally and on the following morning had developed to excellent, large plutei.

It is of interest to compare the results of these experiments with others using the same concentrations and one using 1:2,400,000 solution, in which the results were highly irregular. In the latter concentration, in the diffuse-light culture both cytoplasm and granules stained, the granules becoming bluer than the cytoplasm. In the irradiated cultures the eggs were stained, both cytoplasm and granules staining. In these cultures all the dye was absorbed by the eggs, leaving the solution clear. Cleavage in the cultures, both nonirradiated and irradiated, was irregular, and all the cultures were discarded because of the unsatisfactory development of their sea-water control. There was therefore no evidence that irradiation in the solution of dye had produced any effect.

Other experiments were then performed with 1:600,000 and 1:700,000 solutions of Nile blue A. Inseminated eggs transferred to 1:600,000 solution in sea water 5 minutes after insemination at a temperature of 28.7° C. and kept in diffuse light became slightly greenish in color. They began to enter the 2-cell stage 38 minutes after insemination and developed through a regular, normal cleavage to form fine young plutei. Eggs of the same insemination were transferred to 1:600,000 Nile blue A 5 minutes after insemination and irradiated and stirred for 10 minutes, the temperature rising to 30.4° C. These eggs were stained bluish and showed uniformly distributed blue granules except at the periphery. They cleaved regularly and normally, entering the 2-cell stage somewhat more slowly than those of the diffuse-light culture, but continuing cleavage regularly, and on the following morning had developed to plutei and prism larvae somewhat less advanced than those of the diffuse-light culture.

Inseminated eggs, transferred to 1:700,000 solution of Nile blue A in sea water 5 minutes after insemination and kept in diffuse light at a temperature of 28.9° C., showed faint blue granules. The eggs developed normally in the same tempo as those of the control and on the following morning had developed to fine young plutei. Eggs of the same insemination, transferred to 1:700,000 Nile blue A 5 minutes after insemination, were irradiated and stirred for 10 minutes. At the end of irradiation the culture had reached a temperature of 32.8° C. These eggs were stained very blue, with blue granules. When out of focus the granules were very bright. These eggs developed a little more slowly than those of the diffuse-light control and were entering the 2-cell stage 40 minutes after insemination. The eggs were slightly wrinkled, and 47 minutes after insemination showed a little blister cytolysis. Shortly after entering the 4-cell stage many of these eggs seemed to be developing abnormally, but later most of them became smooth and resumed a normal appearance. On the following morning the control showed some fine young plutei, many prism larvae, and some gastrulae. In the final culture of this series, eggs of the same insemination were transferred to 1:700,000 solution of Nile blue A in sea water 5 minutes after insemination and irradiated and stirred for 20 minutes. The temperature at the end of this time had reached 35.7° C. At the close of irradiation these eggs were stained very blue. The granules were blue and when out of focus very bright, as in the preceding culture. Cytolysis appeared 13 minutes after the end of irradiation, and increased during the next 10 minutes. During the first hour and a half after insemination these eggs failed to divide. There were a few bad 2-cell stages, many dead eggs, and about the same number of blister-cytolized eggs in the culture 2 hours after insemination. On the following day no larvae had developed.

It became evident that even the bright blue 1:700,000 solution of Nile blue A in sea water was a supersaturated solution. The eggs in this solution take up the excess stain more quickly in the irradiated solutions than in the nonirradiated until the solution becomes steel gray in color, this color being the same as that shown by saturated sea-water solutions of Nile blue A made by dissolving the dye directly in sea water. The result of the last experiment, in which the temperature rose to 35.7° C. during irradiation, is convincing in its evidence that increased temperature plus the effect of the dye is more effective than either temperature or dye taken separately. Reference should again be made to the experiment described in the section on irradiated unstained eggs, in which eggs were irradiated and stirred for 20 minutes and the temperature reached 34.8° C. without producing blister cytolysis, the eggs recovering and developing to gastrulae which were normal in appearance.

Cresyl Violet

The cresyl violet used in this work was that of the National Aniline and Chemical Company, certified, dye content 97 per cent. It was used in concentrations of 1:50,000 and 1:100,000.

The first work done was with inseminated eggs which were transferred to 1:50,000 solution 5 minutes after insemination. In the cultures kept in diffuse light the eggs stained blue within 7 minutes; 15 minutes later a few had become extraovate. These eggs entered late anaphase and began to divide 38 minutes after insemination, the insemination-cleavage interval being slightly longer than in the sea-water control. They continued cleavage regularly and formed normal blastulae. Another portion of the same eggs was transferred to 1:50,000 solution of cresyl violet, and irradiated and stirred for 10 minutes. These eggs stained very blue and became slightly irregular in form. This irregularity increased and some eggs became extraovate. A few divided irregularly 40 minutes after insemination. Later many 2-cell stages were formed, the blastomeres becoming rather widely separated. A few eggs continued their division to 4-cell stages with rather irregular blastomeres and some cytolysis. In slightly less than 2 hours after insemination, cleavage stages from 2-cell to 16-cell were to be found in the cultures, the older stages usually with the blastomeres separated from one another into loose groups of cells. At the time when the eggs in the nonirradiated culture had reached the early blastula stage, the irradiated culture consisted mainly of aggregations of cells in the form of a 2-cell stage with many cells in each half.

The next solution tried was of the concentration 1:100,000. Eggs were transferred, 5 minutes after insemination, to a solution kept in diffuse light at a temperature of 31.2° C. These eggs 5 minutes later had a fine spherical form; they were slightly bluish-gray in color and their granules were beginning to stain blue. They began to cleave 35 minutes after insemination and within 41 minutes after insemination most of the eggs were in the 2-cell stage. In 52 minutes after insemination they began to enter the 4-cell stage, and about 20 minutes later the 8-cell stage. These eggs cleaved regularly to 8-, 12-, and 16-cell stages, when they began to cytolize, although cleavage of some of the eggs continued. On the following morning all had disintegrated. Similar inseminated eggs that were transferred to 1:100,000 solution of cresyl violet, and irradiated and stirred for 10 minutes, with the temperature rising during this time to 35.2° C., became very blue during the irradiation and some became superficially roughened. The plasma membrane became thickened and roughened. Many eggs were in blister cytolysis 35 minutes after insemination or 20 minutes after the end of irradiation. About half were blister cytolized 25 minutes after the end of irradiation. None of these eggs divided, and 2 hours later all were cytolized. It should be noted that the temperature in the irradiated culture was excessive.

During the remainder of the work uninseminated eggs were used. In the first of these experiments eggs were transferred to a 1:50,000 solution at 29.3° C. and kept in diffuse light. The eggs were inseminated in this solution 10 minutes later. They stained grayish blue and formed good fertilization membranes. The eggs in the sea-water control began their first cleavage 40 minutes after insemination. The nonirradiated eggs in the solution of dye showed about 10 per cent blister cytolysis, the remainder of the eggs dividing to the 2-cell stage. Uninseminated eggs transferred to 1:50,000 solution, and irradiated and stirred for 10 minutes, the temperature at the end of this time being 29.4° C., were inseminated at the end of irradiation. These eggs stained deep blue. A few formed fertilization membranes. About 1 per cent were in blister cytolysis 25 minutes after the close of irradiation. Blister cytolysis gradually increased, reaching 10 per cent within the next 20 minutes and 15 per cent within 1¼ hours after insemination. Rare 2-cell stages were formed, more than 80 per cent of the eggs remaining spherical, undivided, and unmodified. Uninseminated eggs transferred to 1:50,000 cresyl violet, and irradiated and stirred for 20 minutes, with the temperature controlled and standing at 29.3° C. at the end of irradiation, were inseminated at the close of irradiation. These eggs stained deep blue and showed no fertilization membranes. A few eggs were blistering 5 minutes after the end of irradiation. About 1 per cent were in blister cytolysis 10 minutes later. Blister cytolysis increased, reaching 20 per cent 35 minutes after the end of irradiation and 25 per cent 1 hour and 10 minutes after the close of irradiation. The blisters dropped from these eggs, and the remainder persisted as spherical eggs. Uninseminated eggs were transferred to 1:50,000 solution of cresyl violet, and irradiated and stirred for 20 minutes, with the temperature in the culture held as close to 32° C. as possible (actually 31.8° C.). These eggs were inseminated on removal from the sunlight. They stained deep blue and formed no fertilization membranes, and a few eggs blistered. In 15 minutes after the end of irradiation 10 per cent were in blister cytolysis. Blister cytolysis increased gradually to 35 per cent. Many of these eggs were disintegrating, the blisters dropping from the eggs, 45 minutes after the close of irradiation. The remainder of the eggs retained their spherical form. This series of experiments indicates clearly that increased temperature, even though it is kept at 32° C., increases the violence of the photodynamic reaction.

Two series of experiments were then performed with cresyl violet in 1:100,000 concentration. Uninseminated eggs were transferred to a culture kept in diffuse light at 30.3° C., and after 10 minutes were inseminated in this solution. These eggs stained grayish blue and formed well separated fertilization membranes. In 41 minutes after insemination the eggs were in anaphases of the first division. They cleaved to regular 2- and 4-cell stages and were followed no farther. The eggs closely resembled those of the control. Uninseminated eggs transferred to 1:100,000 solution of cresyl violet, and irradiated and stirred for 10 minutes with the temperature of the culture held at 30.3° C., were inseminated at the end of irradiation. These eggs formed fertilization membranes rarely. The eggs were stained blue and formed 1 or 2 protruding clear vesicles. No blister cytolysis occurred, there was no division, and many of the eggs were dead 2 hours after the end of irradiation. Uninseminated eggs were transferred to 1:100,000 solution of cresyl violet, and irradiated and stirred for 20 minutes, with the temperature held at 30° C. These eggs were inseminated at the close of irradiation. They stained deep blue, formed no fertilization membranes, and showed 1 or 2 protruding clear vesicles. During the next hour no blister cytolysis appeared. Slightly later, many of the eggs died, after becoming irregular, with clear peripheral regions. There was no blistering. Uninseminated eggs transferred to 1:100,000 cresyl violet, and irradiated and stirred for 20 minutes with the temperature held at about 32° C. (actually 31.6° C.), were inseminated at the end of irradiation. During the next 2 hours these eggs resembled those of the preceding culture in every respect.

In the final series of experiments, uninseminated *Lytechinus* eggs were transferred to 1:100,000 cresyl violet, kept in diffuse light at 29.6° C., and inseminated in this solution 12 minutes later. All these eggs formed fertilization membranes. The eggs stained gray. In the sea-water control, cleavage began 39 minutes after insemination. In the diffuse-light culture, it began 42 minutes after insemination. These eggs were followed only to the 4-cell stage. Uninseminated eggs transferred to 1:100,000 solution, and irradiated and stirred for 12 minutes with the temperature held at 29.9° C., were inseminated 12 minutes after the beginning of irradiation. (Clouds covered the sun just as irradiation was begun. There was only sunlight enough to cast a dim shadow. The sun reappeared 7 minutes later.) The eggs stained blue. They formed no fertilization membranes, but a small, protruding, clear vesicle was visible on each egg. On many eggs a closely fitting fertilization membrane was visible 17 minutes later. More than half of these eggs showed the nucleus in mitosis 35 minutes after insemination, although about 1 per cent were in blister cytolysis. These eggs, with the exception of 5 per cent that blistered, formed regular 2's and 4's. Uninseminated eggs transferred to 1:100,000 cresyl violet, and irradiated and stirred for 22 minutes with the temperature held at 29.6° C., were inseminated at the end of irradiation. These eggs formed no fertilization membranes and showed from 1 to 3 small protruding vesicles on each egg. The eggs were bluer than in the preceding culture. Blister cytolysis appeared and gradually increased until 5 per cent of the eggs were affected. There was some division of the eggs to 2-cell and then to 4-cell stages. Uninseminated eggs transferred to 1:100,000 solution of cresyl violet, and irradiated and stirred for 22 minutes at a temperature kept as close as possible to 32° C. (actually 31.8° C.), were inseminated at the close of irradiation. These eggs behaved in all respects like those just described, regular cleavage proceeding to the 2-cell and 4-cell stages; 5 per cent of the eggs blister cytolized.

Results of the Study of Sections of Eggs from Cresyl Violet Cultures

Miss Abbie Ingalls made a study of the effect of nonirradiated solution of cresyl violet on the eggs of *Lytechinus variegatus*. Two strengths of solution, 1:100,000 and 1:50,000, were used. A high majority of the cells were affected in both the weaker and the stronger solution. Eggs from the 1:50,000 solution showed a marked increase over those from the weaker solution in cytoplasmic effects. Many more formed rods, and the number of lobulated eggs as well as the degree of lobulation was greatly increased. Three abnormalities not found in the 1:100,000 solution appeared: complete cytolysis, streak formation, and a type of condensation which seemed to be a rapid, vigorous coagulation of the cytoplasm and which occurred chiefly in cells in which the cytoplasm was in active motion.

Miss Ingalls studied 200 eggs, 100 from each solution. In 31 per cent of the eggs from the weaker solution and in 55 per cent from the stronger solution, small chromosome-like rods appeared in the cytoplasm. The first step in the formation of these rods was the collection of granules either in a group or irregularly aggregated in a line. Associated with these granules was a small condensation of the cytoplasm which formed a matrix in which the granules lay. Sometimes, if the original condensation was large, more than one rod formed in a single condensation. The discrete granules lying in lines fused to form a rod of irregular outline. Finally the outline of the rod became smooth and the matrix disappeared.

Streak formation appeared in 15 per cent of the cells from the stronger solution and in 1 cell only in the weaker solution. The typical streak formation seemed to be the result of the action of the dye on the forming mitotic figure.

In eggs from the weaker solution, only one example of lobulation was found. In the stronger solution, 15 per cent of the eggs studied were lobulated. The nucleus was almost always broken into a number of fragments which formed small secondary nuclei. Often some chromatin remained free in the cytoplasm. There was a tendency for nearly all the eggs to be more condensed at the periphery. Abnormal peripheral condensation may cover a wide area or may occur at a single spot at the periphery. Internal condensation may also occur.

Miss Ingalls found that approximately half the nuclei are abnormal in each group studied. The most common type of abnormality is pycnosis. From 1 to 15 pycnosed chromosomes were found in various cells. The next most common abnormality was lagging of chromosomes. Of the 200 eggs studied, 63 had pycnosed and 15 had lagging chromosomes. Twelve of these 15 eggs also showed pycnosis, and in 8 of these it was the pycnosed chromosome that lagged or was among those lagging.

Occurring less frequently than lagging was chromosome displacement. There were 9 examples of this.

Of very rare occurrence was deflection of the chromosomes. Three examples of this were found.

One per cent blister cytolysis appeared in the eggs from the stronger solution.

All the eggs studied after irradiation were from a 1:100,000 solution. The first of these was irradiated in sunlight for 10 minutes, the temperature of the culture being kept at 29.9° C. About 1 per cent of the eggs showed blister cytolysis; there were 2 or more sperm nuclei in each egg. Anaphases with compact chromosomes were rare.

The second lot of eggs was irradiated for 20 minutes with the temperature cooled to 29.6° C. Most of the nuclei in this culture were intact. There were a few examples of polyspermy, some eggs containing as many as 3 male nuclei. Blister cytolysis was common, reaching 5 per cent.

The final culture was irradiated for 20 minutes, being held at 32° C. during this time. It was of about the same nature as that just described. Polyspermy was very common. In most cases the male and female nuclei were not fused. No normal division was found. Blister cytolysis occurred in 5 per cent of the eggs.

SAFRANIN O

The National Aniline and Chemical Company's safranin O, dye content 97 per cent, certified by the Commission on Standardization of Biological Stains, was used in this work. *Lytechinus* eggs were transferred 5 minutes after insemination to 1:50,000 solution of safranin O in sea water and kept in diffuse light. The nucleus in these eggs was stained pink 7 minutes after transfer to the solution of dye. The eggs were becoming irregular in form 30 minutes after insemination. A few had entered the 2-cell stage 51 minutes after insemination. The control eggs in sea water had reached this stage 38 minutes after insemination. The number of 2-cell stages in the diffuse-light culture increased slowly, but 70 minutes after insemination many irregular 2-cell stages had appeared. When the eggs of the sea-water control had reached a late cleavage stage with a large cleavage cavity, the diffuse-light culture contained only 2-cell, 3-cell, and irregular 4-cell stages and irregular eggs. On the following morning all were dead. A similar diffuse-light culture was prepared in the same way, the eggs being kept in diffuse light at 30.1° C. These eggs cleaved in the tempo of the control to the 64-cell stage. On the following morning all were abnormal blastuiae.

A companion irradiated culture to each of these diffuse-light cultures was also prepared. Eggs transferred to 1:50,000 safranin O 5 minutes after insemination were irradiated and stirred for 10 minutes. The cytoplasm in these eggs was stained brownish pink at the end of irradiation. The eggs in this culture were pinker than those in the diffuse-light culture. They were becoming irregular in form 25 minutes after being placed in the solution of dye, and 2 minutes later the plasma membrane was ruffled. It was not until 86 minutes after insemination that a few irregular 2-cell stages appeared in the culture. Prior to this a few eggs had cytolized. Following this, 3½ hours after irradiation, the culture contained only 2-cell stages, irregular eggs, and cytolizing eggs. On the following morning all were dead; the eggs did not develop beyond the 2-cell stage. The companion to the second diffuse-light culture was prepared by transferring eggs 5 minutes after insemination to 1:50,000 solution of safranin O in sea water and irradiating and stirring for 10 minutes. At the close of irradiation the temperature was 33.6° C. These eggs were slow in entering the first cleavage, but were in 2- and 4-cell stages, with a few cytolized, when the eggs of the diffuse-light culture and the sea-water control were in the 4- and 8-cell stages. Somewhat similarly, they had reached only the 32-cell stage when the eggs of the diffuse-light control were in the 64-cell stage. On the following morning all were abnormal blastulae.

These experiments showed that safranin O was slightly toxic in diffuse-light cultures and that irregular cleavage and cytolysis were produced by irradiation.

MAGDALA RED ECHT

Magdala red echt, Grübler, was used in 1:20,000, 1:40,000, 1:80,000, 1:140,000, and 1:160,000 solutions. In 1:20,000 solutions the irradiated eggs swelled until the diameter of the swollen eggs became half as great again as that of the non-irradiated eggs. These eggs stained pink and the surface formed clear, spherical blisters. In 1:140,000 solution, 3 hours after the end of 15 minutes irradiation, there was 100 per cent cytolysis. In 1:160,000 solutions there was pitting of the surface of irradiated eggs up to 5 per cent and cytolysis in 70 per cent of the eggs, but very little formation of blebs.

The absorption maximum of this dye is close to that of neutral red. The effect of irradiation in solutions of Magdala red echt is somewhat similar to that in solutions of neutral red, the difference being that with Magdala red echt the eggs swell before producing blisters.

A brief summary of the results of the investigation appears in the following table.

Summary of Results of Irradiation of Eggs in Solutions of Magdala Red echt of Various Concentrations for Various Lengths of Time at Various Intensities

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/5/39	(1)	29	1:20,000	0	Dark
	(2)	31.8	1:20,000	10	7969

Results. (1) 5 minutes after insemination no separated membranes, less than 1 per cent pitting and vacuoles; 10 minutes after insemination occasional separated membranes, 2 per cent pitting and vacuolization with inclusions; control beginning 2's 39 minutes after insemination; (1) no 2's 41 minutes after insemination; no granules stained in egg.

(2) No separated membranes; less than 1 per cent pitting and vacuoles 6 minutes after insemination; 9 minutes after insemination occasional separated membranes; 41 minutes after insemination no 2's; 51 minutes after insemination 2 per cent are swollen, stained pink, and disintegrating, diameter of swollen eggs about half as large again as normal eggs; 1½ hours after insemination about 50 per cent of eggs were swollen and stained and showed clear blisters. The absorption maximum of this dye is close to that of neutral red, and the effect of irradiation is somewhat similar to that in neutral red, the difference being that with Magdala red the eggs swell before producing blisters; no granules in the egg were stained.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Light intensity (foot-candles)
7/6/39............	(1)	28	1:20,000	0	Dark
	(2)	30.4	1:20,000	10	1714–1745*
	(3)	30.4	1:20,000	15	1714–1745*

*One 400-watt and one 500-watt lamp.

Results. (1) 6 minutes after insemination no separated fertilization membranes; control in 2's 69 minutes after insemination; (1) 2 hours after insemination no segmentation, no changes evident; on following morning no cleavage had occurred; control, prism larvae.

(2) 6 minutes after insemination no separated fertilization membranes; 70 minutes after insemination no segmentation; 4 hours after insemination no segmentation, no changes evident; on following morning disintegrated, these eggs stained; some not disintegrated, these not stained.

(3) 3 minutes after insemination no separated fertilization membranes; 1 hour after insemination no segmentation, 2 per cent cytolysis; 3 hours and 40 minutes after insemination no cleavage; on following morning disintegrated, these stained; some eggs intact, these unstained.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/7/39............	(1)	27.6	1:20,000	0	Dark
	(2)	29.6	1:20,000	10	4106–4801–5686
	(3)	29.6	1:20,000	15	4106–4801–5686

Results. (1) 3½ minutes after insemination no separated fertilization membranes; 12 minutes after insemination none of eggs have protrusions; 20 minutes after insemination an occasional egg pitted; 43 minutes after insemination no change in eggs evident; 4 hours after insemination no segmentation, eggs intact.

(2) 4 minutes after insemination no separated membranes; 13 minutes after insemination none of eggs have protrusions; 35 minutes after insemination a few eggs show stained spots and disintegration, less than 1 per cent with protrusions, about 1 per cent of eggs

stained pink, enlarged, and beginning to show blisters; 40 minutes after insemination much cytolysis but none like swollen stained eggs in (3); 44 minutes after insemination much irregularity in eggs, disintegration with very little protrusion from surface; 1 hour and 16 minutes after insemination much general cytolysis, some lesser swelling, staining, and blister formation, cytolysis approaching 75 per cent; 4 hours and 25 minutes after insemination no segmentation, 50 per cent of eggs stained pink, cytolysis of stained and unstained eggs 100 per cent.

(3) 3 minutes after insemination no fertilization membranes, about 1 per cent of eggs with slight protrusions; 11 minutes after insemination about 2 per cent of eggs with protrusions, an occasional egg stained pink and swollen; 31 minutes after insemination swelling, staining, and blistering increased, approaching 5 per cent; 34 minutes after insemination swelling, staining, and blistering more than 5 per cent; 40 minutes after insemination swelling, staining, and blister formation about 50 per cent; (3) 55 minutes after insemination, plate 3, figure 1; 68 minutes after insemination swelling, staining, etc., above 75 per cent; 3½ hours after insemination 100 per cent stained and cytolized. Control, plate 3, figure 2.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/8/39............	(1)	28.4	1:40,000	0	Dark
	(2)	30.4	1:40,000	10	8213–9031
	(3)	30.3	1:40,000	15	8213–9031

Results. (1) 4 minutes after insemination no separated membranes; 25 minutes after insemination no change in eggs evident; 1 hour and 25 minutes after insemination no segmentation, pitting 10 per cent; on the following morning most eggs still intact, no segmentation; control, excellent plutei.

(2) 4 minutes after insemination no separated membranes; 11 minutes after insemination more than 10 per cent cytolizing, about 5 per cent stained and swelling; 20 minutes after insemination all eggs cytolizing, 50 per cent stained pink and blistering; 35 minutes after insemination much more blistering than in (3); 36 minutes after insemination large blisters broken from egg; 3 hours and 25 minutes after insemination cytolysis 100 per cent, eggs stained pink and large blisters; on the following morning complete disintegration, masses stained red.

(3) 2 minutes after insemination all eggs cytolizing, stained pink, swollen; 10 minutes after insemination 5 per cent blistering; 31 minutes after insemination all eggs stained and swollen but blistering does not exceed 10 per cent; 58 minutes after insemination eggs have not blistered like (2); 3 hours and 20 minutes after insemination cytolysis 100 per cent, stained pink, few blisters and small; on the following morning cytolysis complete with eggs red, swollen, but intact.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/9/39............	(1)	28.2	1:80,000	0	Dark
	(2)	30.1	1:80,000	10	3984–6317–6641–9297
	(3)	30.0	1:80,000	15	3984–6317–6641–9297

Results. (1) 2½ minutes after insemination some eggs with excellent fertilization membranes; 14 minutes after insemination about 25 per cent with well separated membranes; 31 minutes after insemination both fertilized and unfertilized eggs appear normal; control 40 minutes after insemination constricting; (1) 39 minutes after insemination early anaphase; 40 minutes after insemination fertilized eggs constricting; 44 minutes after insemination all fertilized eggs in or into 2's, unfertilized eggs normal in appearance, unchanged; 51 minutes after insemination 2's and unfertilized eggs; 64 minutes after insemination 2's going to 4's; 2 hours and 11 minutes after insemination 24's and 32's; 5¾ hours after insemination rotating blastulae and undivided, unfertilized eggs; on the following morning somewhat stumpy plutei and undivided, unfertilized eggs.

(2) 3 minutes after insemination no fertilization membranes; 8 minutes after insemination 2 per cent pitted; 19 minutes after insemination 25 to 30 per cent pitted, 2 per cent blistered; 28 minutes after insemination blistering now increased to 25 per cent, most of pitted eggs have now gone into blister cytolysis; 50 minutes after insemination 50 per cent in complete blister cytolysis and stained light pink, remaining eggs swollen slightly but not stained; 2 hours and 14 minutes after insemination 50 per cent in complete blister cytolysis, stained; 50 per cent intact or fragmented, not stained; 6 hours and 14 minutes after insemination disintegration and cytolysis, about 10 per cent of eggs have not stained and have retained their form, some unstained eggs have fragmented; on the following morning complete disintegration.

(3) 2 minutes after insemination 50 per cent pitted; 3 minutes after insemination 50 per cent cytolizing; 7 minutes after insemination cytolizing eggs blistering; 12 minutes after insemination 95 per cent cytolizing, stained only faintly, 80 per cent blistering; 22 minutes after insemination about 5 per cent show poorly separated fertilization membranes; these eggs have not cytolized, but are not normal, are slightly swollen; 43 minutes after insemination about 3 per cent not yet in complete blister cytolysis, 97 per cent stained light pink; 2 hours and 9 minutes after insemination all cytolized; 5 hours and 47 minutes after insemination complete blister cytolysis with all masses of fragments stained; on the following morning complete disintegration.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/11/39.	(1)	28.8	1:140,000	0	Dark
	(2)	30.3	1:140,000	10	8633
	(3)	30.0	1:140,000	15	8633

Results. (1) 3 minutes after insemination 5 per cent with well separated membranes; 21 minutes after insemination about 10 per cent with separated membranes; 36 minutes after insemination constricting for first division; 37 minutes after insemination some in 2's; 65 minutes after insemination 4's and into 4's; 3 hours after insemination hollow spheres; 4 hours and 39 minutes after insemination hollow spheres; 8 hours and 39 minutes after insemination mesenchyme formation; on the following morning plutei with much variation.

(2) 8 minutes after insemination no fertilization membranes, eggs appear normal except there is about 1 per cent pitting; 22 minutes after insemination about 2 per cent blister cytolysis; 28 minutes after insemination 10 per cent pitted and cytolizing; 64 minutes after insemination 10 per cent fragmented with cytolysis, and little blister cytolysis; 3 hours and 5 minutes after insemination 75 per cent cytolized, many with form and color retained; 4 hours and 39 minutes after insemination cytolysis increased; 8 hours and 39 minutes after insemination all cytolizing fragments; on the following morning complete disintegration.

(3) 1 minute after insemination eggs normal in appearance; 5 minutes after insemination no separated membranes, about 2 per cent pitting and beginning cytolysis; 20 minutes after

insemination 75 per cent cytolizing, 50 per cent blistering, these latter being swollen and light in color; 38 minutes after insemination about 90 per cent cytolized, most with blisters; 58 minutes after insemination 95 per cent cytolized, 50 per cent have blisters; 3 hours and 7 minutes after insemination 100 per cent cytolized, nearly colorless; 4 hours and 35 minutes after insemination all masses of cytolized fragments stained pink; 8 hours and 35 minutes after insemination all cytolizing, stained, some irregular, some spherical forms; on the following morning all showed complete disintegration.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/10/39............	(1)	28.9	1:160,000	0	Dark
	(2)	30.0	1:160,000	10	4738–5047–5312–6641
	(3)	30.1	1:160,000	15	4738–5047–5312–6641

Results. (1) 2 minutes after insemination excellent fertilization membranes; 14 minutes after insemination 90 per cent fertilized; 34 minutes after insemination early anaphases; 37 minutes after insemination many in constriction; 39 minutes after insemination, into 2's; 50 minutes after insemination 90 per cent in 2's, 10 per cent unfertilized; 1 hour and 21 minutes after insemination excellent 4's; 3 hours after insemination hollow spheres like control; 5¾ hours after insemination blastulae beginning to swim; on the following morning excellent plutei.

(2) 3 minutes after insemination no fertilization membranes; 13 minutes after insemination 2 per cent pitted; 21 minutes after insemination not more than 2 per cent pitted, pits (cups) and vacuoles contain concretions; 41 minutes after insemination pitting increased; 78 minutes after insemination an occasional 2-cell stage (less than 1 per cent), with poorly separated fertilization membrane; 3 hours and 10 minutes after insemination 15 per cent cytolysis, mostly in irregularity of form with some fragmentation; 5¾ hours after insemination 40 per cent cytolizing, an occasional pitted 2-cell stage; on the following morning disintegrated.

(3) 1 minute after insemination 5 per cent pitted; 3 minutes after insemination 5 per cent pitted; 6 minutes after insemination 5 per cent pitted; 14 minutes after insemination pitted eggs cytolizing, others smooth; 37 minutes after insemination about 7 per cent cytolysis; 39 minutes after insemination cytolysis increasing, cytolizing eggs slightly pink, very little (less than 1 per cent) bleb formation; 49 minutes after insemination cytolysis more than 10 per cent, less than 1 per cent bleb formation; 69 minutes after insemination mock-orange-like forms now appearing to the extent of 2 to 3 per cent; 3 hours after insemination 60 per cent cytolizing, a good deal of variation, some blistering, some simple extreme irregularity of form; 5 hours and 39 minutes after insemination 70 per cent cytolizing; on the following morning all were disintegrated.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/12/39............	(1)	29.2	1:160,000	0	Dark
	(2)	29.5	1:160,000	10	5977
	(3)	29.5	1:160,000	15	5977

Results. (1) 2 minutes after insemination some well separated membranes, most eggs not fertilized; 25 minutes after insemination not more than 5 per cent with fertilization membranes; 38 minutes after insemination, into 2's; 5 hours and 27 minutes after insemination swimming blastulae; on the following morning prism larvae and unfertilized eggs that have disintegrated; control, prism larvae and young plutei.

(2) 3 minutes after insemination no separated membranes; 24 minutes after insemination 1 to 2 per cent cytolizing, form retained; 40 minutes after insemination 10 per cent cytolysis, 5 per cent irregular and fragmented (this 5 per cent is included in the 10 per cent); 5 hours and 29 minutes after insemination eggs stained red, disintegrated and with blisters, unstained eggs fragmented and misshapen; on the following morning these eggs like (3), but some eggs have retained their normal form and color.

(3) 2 minutes after insemination 10 per cent pitted with concretions; 3 minutes after insemination 20 per cent pitted and beginning to cytolize; 10 minutes after insemination 20 per cent in blister cytolysis; 14 minutes after insemination cytolysis increased; 17 minutes after insemination 75 per cent cytolized and disintegrating; 5 hours and 26 minutes after insemination nearly all stained, cytolized masses with blisters, a few unstained fragmented eggs; on the following morning all cytolized, stained red; red core and thick cortex of pink globules.

NIGROSIN

The dye used in these experiments was the Coleman and Bell Company's nigrosin, water soluble, certified by the Commission on Standardization of Biological Stains.

In the first experiments nigrosin was used in 1:150,000 concentration. Inseminated *Lytechinus* eggs, transferred to a solution of this concentration in sea water 5 minutes after insemination and kept in diffuse light, began to enter the 2-cell stage 46 minutes after insemination. Most of these eggs had completed the second division 28 minutes later, or 74 minutes after insemination. Inseminated eggs from the same lot, transferred to 1:150,000 nigrosin in sea water and irradiated and stirred for 20 minutes, usually did not divide. A few 2-, 3-, and 4-cell stages were present in the culture 74 minutes after insemination. These eggs were not stained and no photodynamic effect was evident.

The next experiment was done with nigrosin in 1:75,000 concentration. Inseminated eggs were transferred to solutions of nigrosin of this strength in sea water 6 minutes after insemination and kept in diffuse light. They began to enter the 2-cell stage 41 minutes after insemination. Inseminated eggs from the same lot were transferred to solution of nigrosin 6 minutes after insemination and irradiated and stirred for 20 minutes. These eggs were entering the 2-cell stage in 40 minutes. Possibly the slight increase in temperature during irradiation hastened cleavage. No photodynamic effect on the eggs was evident.

As neither of these solutions showed photodynamic effect, a still stronger solution, 1:20,000, was used, and sea-water solutions of this concentration were used throughout the remaining experiments.

Inseminated *Lytechinus* eggs were transferred to 1:20,000 solution of nigrosin in sea water 5 minutes after insemination and kept in diffuse light. They began to enter the 2-cell stage 37 minutes after insemination, and continued their cleavage regularly during the next 5 hours. Eggs of the same insemination were transferred 5 minutes after insemination to 1:20,000 nigrosin in sea water and irradiated and stirred for 20 minutes. Thirty-seven minutes later they had become irregular in shape and were beginning to divide into irregular 2-cell stages. At this time some of both the undivided and the divided eggs began to show cytolysis. Most of the eggs had reached an irregular 2-cell stage 4 minutes after division began. As cleavage proceeded, many eggs with 1 of the first 2 cells cytolized, the other cleaving, were seen. The eggs of this culture showed considerable diversity after 4 hours development. There were some eggs in which both of the first 2 blastomeres had cleaved independently, some eggs which were completely cytolized, and some which were half good and half cytolized. In the next experiments the effect of greatly increased temperature during irradiation was tried. The solutions of nigrosin were deep purple in transmitted light and black in reflected light. These exceedingly dark solutions absorbed heat strikingly during irradiation. For the diffuse-light culture inseminated eggs were transferred to 1:20,000 solution of nigrosin in sea water 5 minutes after insemination at a temperature of 29.6° C. These eggs showed sharp contours 4 minutes after being placed in the solution, and 4 minutes later showed radiations in the cytoplasm surrounding the nucleus. Many of these eggs had divided to 2 cells 38 minutes after insemination, and most of the others were in anaphases of the first division. All these eggs divided regularly to 2-, 4-, and 8-cell stages and continued to normal late cleavage stages with a large cleavage cavity.

The irradiated cultures were two in number. For the first culture, inseminated *Lytechinus* eggs were transferred to 1:20,000 solution of nigrosin in sea water, and irradiated and stirred for 10 minutes, during which the temperature reached 34.2° C. These eggs 4 minutes after the end of irradiation were unstained and their contours were good. The eggs remained sharp in outline and were entering the 2-cell stage 30 minutes after insemination. Most were in good 2-cell stages 5 minutes later. This extremely rapid first division must be attributed to the high temperature. The eggs continued to cleave to 2-, 4-, and 8-cell stages, cleavage being irregular. Great variation in the stages of development from the 2-cell stage to late cleavage was evident in this culture 2½ hours after insemination. At this time the late cleavage stages were not quite so far advanced as those of the diffuse-light culture, the temperature of which had never been raised above 29.6° C.

For the final culture of this series, inseminated eggs were transferred to 1:20,000 solution of nigrosin in sea water and irradiated and stirred for 15 minutes. The temperature had reached 36.1° C. at the end of irradiation. These eggs were unstained and their contours were slightly irregular 1 minute after the close of irradiation. All the eggs were irregular in form 20 minutes later. Many of the eggs were blister cytolizing and none were dividing 20 minutes after the end of irradiation. Many were blister cytolized but some had become smooth 7 minutes later. None of these eggs had divided an hour after the end of irradiation. All were dead and disintegrating 2 hours after the end of irradiation. Obviously the temperature in the irradiated cultures was too high.

These experiments were repeated at about the same temperature. Eggs from three *Lytechinus* females were mixed, too few being obtained from any one individual, and inseminated. Some of these eggs were transferred to 1:20,000 solution of nigrosin in sea water 5 minutes after insemination and kept in diffuse light. The temperature of this culture was 27.2° C. These eggs were unstained 7 minutes after being placed in the solution of dye, but 7 minutes later showed faintly stained grayish-blue granules throughout the egg. Cleavage in this culture began 40 minutes after insemination. It proceeded regularly to the 32-cell stage in the same tempo as the sea-water control, reaching this stage 2 hours and 20 minutes after insemination.

Inseminated eggs of the same lot were transferred to 1:20,000 solution of nigrosin in sea water and irradiated and stirred for 15 minutes. At the close of irradiation the temperature had reached 35.2° C. The eggs were spherical and showed grayish-blue granules. These eggs were dividing into irregular 2-cell stages 37 minutes after insemination. They were cytolizing slightly along the division furrow 7 minutes later. Some of the undivided eggs were irregular in form and were cytolizing. The culture contained, 53 minutes after insemination, undivided eggs, 2-cell stages, disintegrating eggs, and some dividing eggs with one or both blastomeres disintegrating. The culture contained, an hour and a half after insemination, about 50 per cent undivided eggs, and 2-cell, 4-cell, and a few 8-cell stages. All the 8-cell stages were irregular. Later this culture contained stages from that of the undivided egg to the 16-cell stage, showing great variation and irregularity.

Some of the same lot of inseminated eggs were transferred to 1:20,000 solution of nigrosin in sea water 5 minutes after insemination, and irradiated and stirred for 20 minutes; the temperature at the close of irradiation was 36.8° C. The eggs of this culture were slightly rough at the end of irradiation. They had an intact nucleus 1 hour and 20 minutes after insemination; most of the eggs showed slight cytolysis. These eggs were kept under observation for 2 hours and 20 minutes after insemination and during this time showed no cleavage.

It is evident from the greatly increased temperature in the irradiated cultures that the black solutions absorbed heat rapidly. The temperatures (35.2° C. and 36.8° C.) were too high for normal development. This experiment was therefore repeated with the temperature controlled at a lower level.

The temperature during the following experiments was controlled in the irradiated cultures by stirring with a test tube filled with ice water. *Lytechinus* eggs from two females were mixed and inseminated. Some of these eggs were transferred to 1:20,000 solution of nigrosin in sea water 5 minutes after insemination and the culture kept in diffuse light at a temperature of 28.2° C. These eggs were spherical in form. They were beginning to enter the 2-cell stage 34 minutes after insemination, the insemination-cleavage interval being the same as in the sea-water control. The eggs continued their cleavage regularly and at the end of 1 hour and 53 minutes had reached the 16-cell stage. On the following morning the larvae were living, swimming, normal late gastrulae. Eggs of the same insemination were transferred to solution of nigrosin in sea water, 1:20,000, 5 minutes after insemination, and irradiated and stirred for 15 minutes. The controlled temperature at the end of this time was 27.4° C. The eggs in this culture were entering the 2-cell stage 34 minutes after insemination. They continued their cleavage regularly in the same tempo as the eggs of the sea-water control and of the diffuse-light culture. On the following morning they were living, normal late gastrulae slightly less advanced than in the two preceding cultures. For the final culture in nigrosin, inseminated *Lytechinus* eggs were transferred 5 minutes after insemination to 1:20,000 solution in sea water and irradiated and stirred for 20 minutes. The controlled temperature at the end of this time was 27.6° C. The eggs of this culture remained spherical and were entering the 2-cell stage 34 minutes after insemination. They continued cleavage regularly, reaching the 16-cell stage synchronously with the other cultures described. On the following morning they were late gastrulae similar to those of the preceding culture, but slightly less advanced than those of the control and of the diffuse-light culture.

It may be noted that in all these cultures, including the control, there were eggs showing slight cytolysis after the first cleavage had been completed. All the eggs improved in appearance before the beginning of the second cleavage.

Results of the Study of Sections of Eggs from Nigrosin Cultures

In the control eggs the centers were large; in some instances the chromosomes were sharply stained, in others they were diffusely stained. There was slight lagging of the chromosomes; there were no globules scattered in the cytoplasm.

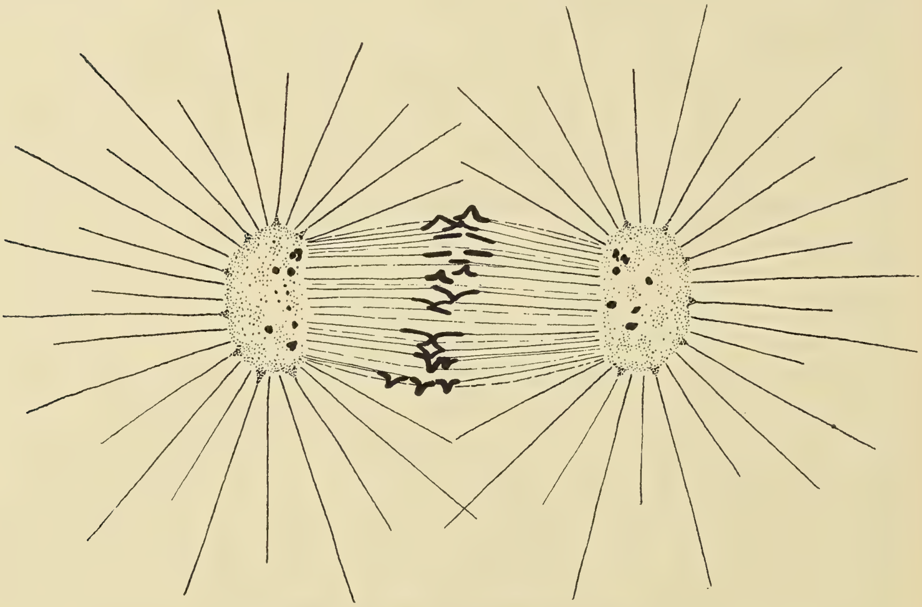


Fig. 14. Nigrosin, 7/27/36, 4:13 p.m.

In agreement with the control, the chromosomes of the nonirradiated eggs in 1:20,000 solution of dye were either sharply stained or diffusely stained. There was slight lagging of chromosomes in their approach to the poles, and there were no globules in the cytoplasm. In these eggs there was usually a small granule in contact with the nucleus; sometimes there were two such granules.

In eggs that had been irradiated for 10 minutes in 1:20,000 solution of nigrosin, in the telophases there might be as many as ten large, deeply stained globules in the interzonal region. These globules sometimes formed deeply stained clumps at the edges of the spindle. The anaphase figures (fig. 14) showed that the chromosomes separate slowly, although the figures had a normal appearance.

Auramin O

Auramin O was used in concentrations of from 1:150,000 to 1:5,000,000. The experiments cover work with both pre-inseminated and post-inseminated eggs. The dye was found to be exceedingly toxic in nonirradiated as well as in irradiated cultures.

The first concentration used was a 1:150,000 solution in sea water of Coleman and Bell's auramin O. Inseminated eggs transferred to a solution of this strength 6 minutes after insemination and kept in diffuse light, like inseminated eggs in cultures irradiated and stirred for 10 minutes, stained a striking lemon yellow. The fertilization membranes began to slip from these eggs about 15 minutes after the end of irradiation. The eggs remained undivided, and in many it could be seen that the egg and sperm nuclei had not united, in other words, fertilization had not been completed. No development took place, and the following day the cultures were discarded.

Experiments with 1:300,000 solutions were then performed. Eggs with excellent fertilization membranes were transferred to 1:300,000 solutions 10 minutes after insemination. In the nonirradiated cultures the eggs stained light yellow and the fertilization membranes remained intact. About 1 hour and 40 minutes after insemination many eggs showed fragmentation of the nucleus, although no normal mitotic figures could be seen. During the ensuing 2 hours the eggs with fragmented nuclei divided to form irregular clumps. These eggs underwent no further development.

In the eggs transferred 10 minutes after insemination to 1:300,000 solution and irradiated and stirred for 10 minutes, the cytoplasm stained a more vivid lemon yellow than in eggs in the nonirradiated cultures. The fertilization membranes remained intact. During the first 20 minutes after the end of irradiation many of the eggs became yellower and some developed irregular contours. The nucleus remained intact and became larger than in the nonirradiated eggs. These eggs remained unchanged except for an increase in intensity of the yellow color. It should be noted in passing that these eggs were not transferred to the solution of auramin O until after the germ nuclei had united in fertilization.

When it became evident that the 1:300,000 solution was too toxic, a 1:600,000 solution was tried. Eggs were transferred to solutions of this strength 10 minutes after insemination. The eggs stained slightly yellow. Ten minutes after the transfer, radiating lines were visible in the cytoplasm surrounding the nucleus; these lines had disappeared 10 minutes later. The eggs remained undivided.

Eggs transferred to 1:600,000 solutions 10 minutes after insemination and irradiated and stirred for 10 minutes were yellower than the nonirradiated eggs. They showed no nuclear activities and underwent no divisions.

Work with 1:1,200,000 concentrations of auramin O was then undertaken. In the first of these experiments, eggs were transferred to the solutions of auramin O 10 minutes after insemination. The nonirradiated eggs stained slightly yellow and looked as if their development had stopped. Forty-eight minutes after insemination, however, some were going into irregular 2-cell stages. These were 12 minutes slower than the eggs in the sea-water control. Many eggs had entered or were entering the 2-cell stage 50 minutes after insemination. Within 3 hours after insemination none of the eggs showed more than very irregular cleavage, and on the following day all were dead, none having formed blastulae.

Inseminated eggs irradiated and stirred for 10 minutes after being transferred to the solution of auramin O were yellower than the nonirradiated eggs. These eggs showed a few retarded and modified anaphase figures, but formed no 2-cell stages. They had broken down 3 hours after insemination, forming masses that resembled the results of irregular cleavage. They never gave rise to embryos.

In the next experiment the sunlight failed when a shower came up about 2 minutes after the irradiation began. The results in the diffuse-light cultures agreed with those previously obtained; those derived from the irradiated cultures were not satisfactory, but because they were significant and in accord with those found under more favorable conditions, they are included here. *Lytechinus* eggs were transferred to 1:1,200,000 solutions of auramin O 10 minutes after insemination. In the sea-water control, eggs were entering the 2-cell stage 40 minutes after insemination. It was not until 65 minutes after insemination that a few irregular divisions appeared in the nonirradiated cultures in auramin O. These eggs continued in very irregular cleavage, all the eggs dying within 12 hours.

The eggs transferred to 1:1,200,000 solutions 10 minutes after insemination, which were to have been irradiated 10 minutes but which were actually irradiated only 2 minutes, showed no divisions at the time when a few irregular divisions were appearing in the nonirradiated eggs. Cytolysis appeared in many a little later. Many remained unmodified. All died within 12 hours.

An experiment with 1:2,400,000 solution of auramin O was being conducted at the same time and, having been subject to the same weather conditions as the experiment just described, is also included. The eggs transferred to 1:2,400,000 solution 10 minutes after insemination and not irradiated began to enter the 2-cell stage 2 minutes later than did those in the sea-water control. These eggs cleaved somewhat irregularly, and on the following day, when the control showed excellent plutei, had given rise to blastulae filled with cells, and some imperfect and incomplete gastrulae.

The eggs transferred to 1:2,400,000 solutions 10 minutes after insemination, and irradiated and stirred for 2 minutes, showed a few irregular 2-cell stages 40 minutes after insemination. Many eggs completed the first division irregularly and continued to about the 16-cell stage. All were dead 12 hours later.

In the experiments of the day following there was a similar interruption, and because of this, reports of experiments with 1:1,200,000 and 1:2,400,000 solutions will be presented together. In both these experiments sunshine was interrupted by rain for 5 minutes. The irradiated cultures were, therefore, given a 5-minute irradiation with sunlight, then a 5-minute irradiation with diffuse light, and finally another 5-minute irradiation with sunlight, a total of 10 minutes irradiation with sunlight. Eggs in the sea-water control were entering the 2-cell stage 38 minutes after insemination. Some nonirradiated eggs in 1:1,200,000 solutions of auramin O showed modified anaphase figures 41 minutes after insemination and were dividing badly 43 minutes after insemination. These eggs were followed for about 6 hours, when eggs in the sea-water control had reached the stage of swimming blastulae with 4 to 8 mesenchyme cells. The experimental eggs had formed during this time only irregular clumps of somewhat dubious cells. Some, but not all, of the spherules of protoplasm seemed to be nonnucleated. The eggs transferred to 1:1,200,000 solution 10 minutes after insemination and given the interrupted 10 minutes irradiation with sunlight, during which they were stirred, showed a modified nucleus, but none of the eggs divided normally. About 10 per cent of these eggs had formed irregular clumps of cells (?) 3 hours after insemination. In their further development these aggregates differed from those in the nonirradiated culture in consisting of larger cells or fragments.

The eggs transferred to the 1:2,400,000 solution 10 minutes after insemination showed many poor 2-cell stages 47 minutes after insemination. Most of the eggs in this culture had entered the 2-cell stage, some being good, 58 minutes after insemination. In their continued development, the cleavage of the eggs in this culture was bad, the final result being irregular clumps of cells.

Eggs transferred to 1:2,400,000 solution 10 minutes after insemination and given the interrupted 10 minutes irradiation with sunlight did not divide normally, although all showed several nuclei, many showing eight. These eggs gave rise to irregular clumps of cells (?), most of which cytolized.

The work with 1:1,200,000 solutions was concluded by experiments with exceedingly favorable material conducted under exceptionally good conditions. *Lytechinus* eggs 10 minutes after insemination were transferred to a 1:1,200,000 solution of auramin O and kept in diffuse light. The eggs in the sea-water control were nearly all in the 2-cell stage 44 minutes after insemination. A few eggs in the diffuse-light dye culture were beginning to enter the 2-cell stage 46 minutes after insemination. Nearly all the eggs in this culture showed no cytoplasmic division 1 hour after insemination, but had two nuclei of the same size.

Eggs transferred to a 1:1,200,000 solution 10 minutes after insemination, and irradiated and stirred for 10 minutes, showed no indication of division until 57 minutes after insemination, when two nuclei could be seen in a very few eggs, there being, however, no division of the cytosome. Eggs in the sea-water control were in the 4-cell stage at this time. As time passed, the nucleus elongated in some eggs, but in most eggs no other change was evident. The development was followed for about 3 hours. At the end of this time there was some irregular division, but in most eggs the nuclei looked fragmented and there had been no division of the cytosome.

In the final experiment with cultures in 1:1,200,000 solutions of auramin O, the eggs were studied with a 4-millimeter apochromatic objective and a 10× compensating ocular. Eggs were transferred 10 minutes after insemination to a diffuse-light culture. Prophases appeared in these eggs 31 minutes after insemination, and anaphases 11 minutes later. The asters were very close together. Some of the eggs divided irregularly 47 minutes after insemination; many more had divided badly 8 minutes later, giving rise to irregular cells with rough, irregular protrusions. Most of the undivided eggs showed, about 15 minutes later, two complete mitotic spindles with asters. The two amphiasters were sometimes connected with each other at one or both poles. There was a suggestion of additional cytasters, but these could not be made out clearly. Each of these eggs, 3 hours later, consisted of a mass of irregular spherules (cells?).

The eggs transferred to 1:1,200,000 solution 10 minutes after insemination, and irradiated and stirred for 10 minutes, showed no indication of development during the first 50 minutes after insemination. Poorly defined cytasters were visible and the nucleus was still intact 1 hour and 20 minutes after insemination. During the next hour most of the eggs cytolized badly, although some looked like irregular 2-, 3-, and 4-cell stages.

Most of the work done with cultures in 1:2,400,000 auramin O has been reported in connection with work done with stronger solutions. There remain, however, two series of experiments that have not yet been described. *Lytechinus* eggs were transferred 10 minutes after insemination to 1:2,400,000 solutions of auramin O. The eggs in the nonirradiated cultures formed spindles that seemed lacking in asters. Many formed good anaphases but began dividing badly to very ragged 2-cell stages 41 minutes after insemination. Half an hour later, or 1¼ hours after insemination, the eggs in this culture were either poor 2-, 3-, and 4-cell stages, or undivided eggs with two nuclei. They had become irregular 16- and 24-cell stages 2¼ hours after insemination.

The eggs transferred to 1:2,400,000 solution of auramin O 10 minutes after insemination and then irradiated and stirred for 10 minutes showed no normal mitotic figures during the first 1½ hours after insemination. There were, however, some modified figures, and there was some nuclear division, two nuclei appearing in many eggs 41 minutes after insemination. The number of multinucleate eggs increased during the next 10 minutes. Some of these eggs divided or partially divided later, the culture finally containing 2-, 4-, and 8-cell stages, irregular clumps of cells, and cytolized eggs.

In the final work with a solution of this concentration, the eggs were first stained and irradiated in a solution of auramin O in sea water and were then transferred to a 1:150,000 solution of neutral red in sea water. For the cultures in diffuse light, eggs were transferred 10 minutes after insemination to the solution of auramin O and 27 minutes later to the solution of neutral red. Most of these eggs entered the 2- and 4-cell stages in approximately the same time as those in the sea-water control. They were followed no farther.

Eggs transferred to the solution 10 minutes after insemination were irradiated and stirred for 10 minutes and then transferred to 1:150,000 solution of neutral red 37 minutes after insemination. In these eggs the neutral red granules stained well and there were some irregular divisions. These eggs also were followed no farther.

It is evident that in all the experiments thus far described, toxic effects followed all the concentrations of auramin O. A solution of lower concentration, 1:4,800,000, was therefore tried. This solution was so slightly yellow that it was distinguishable from sea water only when compared directly. Eggs which were transferred 10 minutes after insemination to this 1:4,800,000 solution showed, 25 minutes after insemination, an apolar spindle stretching straight across the egg. These eggs were entering the 2-cell stage 40 minutes after insemination and produced very good 2-cell stages which continued regularly through successive stages to the 24-cell stage and beyond.

Eggs that were transferred 10 minutes after insemination to the solution of auramin O, and irradiated and stirred for 10 minutes, usually showed no spindle, although they did show a modified anaphase figure. These eggs also were entering the 2-cell stage 40 minutes after insemination. They formed successively very irregular 2-, 4-, 8-, and 16-cell stages.

In the next experiment, eggs that were transferred to 1:4,800,000 solution of auramin O 10 minutes after insemination, and irradiated and stirred for 10 minutes, cleaved irregularly, giving, however, a few good 2-cell stages. It is evident that the development of nonirradiated eggs in 1:4,800,000 solutions approached the normal more closely than in any of the stronger solutions.

In the final work with solutions of approximately this strength, uninseminated *Lytechinus* eggs were transferred to a solution of 1:5,000,000 concentration, kept in diffuse light at a temperature of 27.9° C., and inseminated in this solution 10 minutes later. All the eggs formed excellent fertilization membranes. There was some irregularity in the reaction of the eggs to the stain, some becoming much more yellow than others. The first constriction appeared 41 minutes after insemination. Cleavage was somewhat slow, and it was not until nearly 20 minutes later that most of these eggs had entered the 2-cell stage.

Three different cultures of uninseminated, irradiated eggs were followed. The first of these was irradiated for 10 minutes, the culture being cooled and held at 27.9° C. during irradiation. These eggs were inseminated immediately after the completion of irradiation in the solution in which they had been irradiated. The eggs formed excellent fertilization membranes. Their staining was uniform in color. Their cleavage was modified. Their nuclei had divided without division of the cytosome 48 minutes after insemination. Some eggs had reached an effective 2-cell stage 10 minutes later.

Uninseminated eggs irradiated and stirred for 20 minutes with the temperature reduced to 28° C., and inseminated immediately after the end of irradiation, formed fertilization membranes less well separated than in the two cultures just described. Somewhat rare cytolysis became evident in these cultures 20 minutes after insemination. Many eggs showed two nuclei without division of the cytosome 43 minutes after insemination. During the time that this culture was followed, no division of the cytosome occurred.

Uninseminated eggs that were irradiated and stirred for 20 minutes in a 1:5,000,000 solution of auramin O in which the temperature was permitted to rise, but was kept below 32° C. (actually 31.8° C.), were inseminated immediately after the close of irradiation. These eggs formed fertilization membranes that were less well separated than in the immediately preceding cultures. A good deal of fine blistering appeared within 15 minutes after insemination. This was followed by a greatly increased blistering and by blister cytolysis, the original blistered eggs meantime having disintegrated.

It is evident that the dye auramin O is exceedingly toxic even in solutions in diffuse light. It is further evident that with solutions of lower concentration a photodynamic effect follows irradiation with sunlight. It is also evident that increase of temperature to approximately 32° C. increases the photodynamic effect.

A few additional experiments were performed with auramin O in 1:5,000,000 concentration to which sufficient neutral red was added to make the concentration of neutral red 1:150,000. Uninseminated *Lytechinus* eggs, inseminated after 10 minutes in a diffuse-light culture of this mixture at a temperature of 29.2° C., formed excellent fertilization membranes. These eggs developed hernia-like protrusions 15 minutes after insemination, the hernias becoming rather deeply dyed with neutral red. This culture finally showed rare normal 2-cell stages, a few eggs that were very imperfectly constricted, and some spherical undivided eggs, but most of the eggs formed highly irregular clumps of cells.

Uninseminated eggs that were irradiated and stirred for 10 minutes in this mixture, cooled and held at 29.2° C. during irradiation, and inseminated at the close of irradiation formed no fertilization membranes, but a little blister appeared at the point where the spermatozoon may have entered. The surface of some of those eggs that were smooth during the first 15 minutes after insemination became rough and then within the next 10 minutes showed blister cytolysis, which gradually increased in extent until an hour and a half later it involved 35 per cent of the eggs. The remainder of the eggs were smooth, with intact nuclei. No cleavage occurred.

Another culture of uninseminated eggs was irradiated and stirred in the mixture of auramin O and neutral red for 20 minutes, the temperature being kept at 29.2° C. These eggs were inseminated at the close of irradiation. They were then smooth and formed no fertilization membranes. Ten minutes later many eggs became rough, some with a clear periphery. After another 10 minutes, or 20 minutes after insemination, all became irregular, some with clear peripheral spots, some with definite blisters. About 40 minutes after insemination most of the eggs had entered lobular cytolysis and began to disintegrate. Lobular disintegration became almost complete within the next hour, although some colorless cytolysis also occurred.

In the final experiment in this series, uninseminated eggs were irradiated and stirred in the dye mixture for 20 minutes at a temperature kept at 32° C. (actually 31.7° C.). These eggs were inseminated at the end of irradiation. No fertilization membranes were formed. The surface of some of the eggs became rough within 2 minutes after insemination. All became more or less rough, some with clear spots, some with a blistered surface, within the next 10 minutes. The eggs remained in this condition for the next 30 minutes, when lobular cytolysis began. These eggs all died without cleaving before lobulation became complete. There was considerable modified blistering. It is clear that the increase in temperature to 31.7° C. increased the violence of the photodynamic effect.

The facts presented show that when eggs, immediately after insemination, are placed in solutions of auramin O that are too toxic, or in solutions that become too toxic upon irradiation, union of the germ nuclei does not take place and the eggs retain a normal contour and undergo no further development.

When the processes of fertilization are completed before the eggs are placed in dilute solutions of auramin O and irradiated, the photodynamic effects are on the processes of mitotic division; the spindle is greatly reduced in size and is usually multipolar, and division of the nucleus is not followed by division of the cytosome. In the normal division of this sea-urchin egg the division figure fills the cell, and the radiations from the poles of the spindle extend to the surface of the egg. In the modified development just described, the spindle is reduced in size to about the length of the diameter of the nucleus, and in most cases normal radiations from the poles are completely suppressed. The failure of the mitotic figure to elongate seems to be associated with the lack of asters in the division figures. The two daughter nuclei when formed may lie in contact or at most be separated only slightly. The multipolar spindles result in the formation of many partial nuclei, that is, nuclei containing only part of their chromosomal complement. It seems, in many instances, that fragmentation of the nucleus may be occurring. Evidence both for and against the occurrence of this phenomenon will be presented later.

Uninseminated eggs irradiated in solutions of auramin O and fertilized after removal to diffuse light show the same reactions as eggs that are not irradiated until after insemination.

Results of the Study of Sections of Eggs from Auramin O Cultures

The first material to be presented will be that of the dye control cultures, that is, the nonirradiated cultures in solutions of auramin O in sea water, reared in diffuse light. Deviations from the normal in these must be regarded as specific effects of the dye, not as photodynamic effects.

Eggs from five nonirradiated cultures were fixed, sectioned, stained, and studied. As noted earlier in this paper, the characteristic effects of auramin O are exhibited within the egg, and not as a surface reaction.

A deviation from the normal that may be regarded as mild may be produced by concentrations of 1:5,000,000, although it is also formed occasionally after the use of solutions as strong as 1:1,200,000, the effect of which is usually more violent. This effect of the dye is shown by the achromatic mitotic figure, which appears as a short, anastral spindle, little longer than the diameter of a normal nucleus (fig. 15). On this short spindle the usual phases of mitotic division occur, and the segmentation nucleus may be divided to two daughter nuclei of equal size, which remain close together or actually in contact with each other. Division of the cytosome does not accompany division of the nucleus, and the occurrence of undivided eggs with two nuclei of equal size is common.

The failure of the central spindle to elongate, like the failure of astral rays to develop and the failure of the cytosome to divide, are probably to be attributed to a physical change in the cytoplasm, possibly of the nature of a greatly increased density. It is to this fundamental condition rather than to the lack of

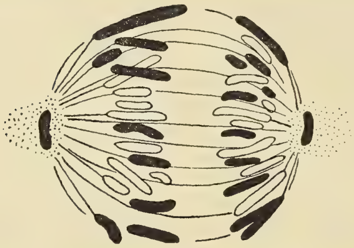


Fig. 15. Auramin O, 8/17/35, 11:23 a.m.

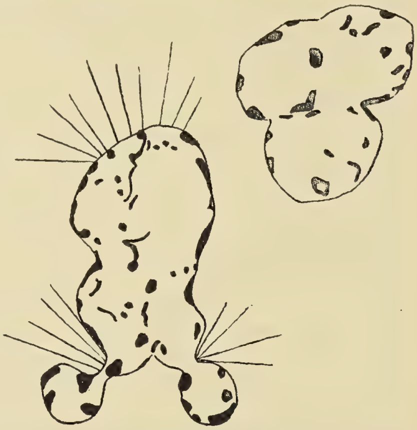


Fig. 16. Auramin O, 8/21/35, 5:40 p.m.

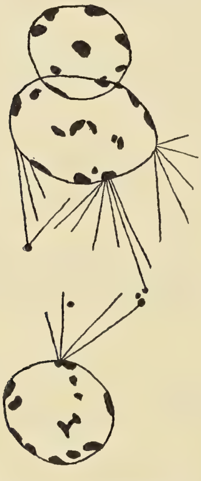


Fig. 17. Auramin O, 8/21/35, 5:40 p.m.

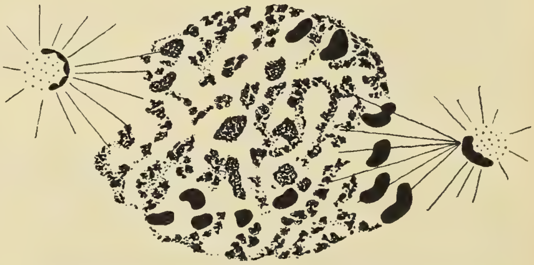


Fig. 18. Auramin O, 8/22/35, 10:40 a.m.

asters that failure of the spindle to elongate and of the cytosome to divide must be attributed.

In inseminated eggs kept in 1:1,200,000 solution of auramin O in sea water in diffuse light, there was often the suggestion of fragmentation of the nucleus to chromosomal vesicles (figs. 16, 17). In other eggs there were well defined centers, a cone of rays extending from each center into the disintegrating nucleus (fig. 18). This condition was followed by one in which elongated chromosomes lay on the spindle. In rare instances the mitotic figure was complete (figs. 19, 20); there were clear centers with rays extending to the surface of the egg. This was exceptional. In most instances the nucleus lay in a mass of hyaloplasm from which lines radiated in all directions.

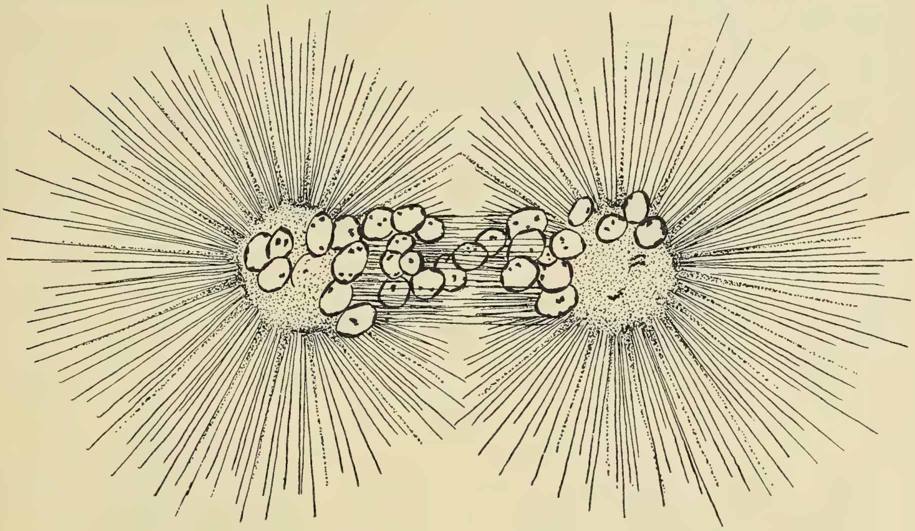


Fig. 19. Auramin O, 8/22/35, 10:51 A.M.

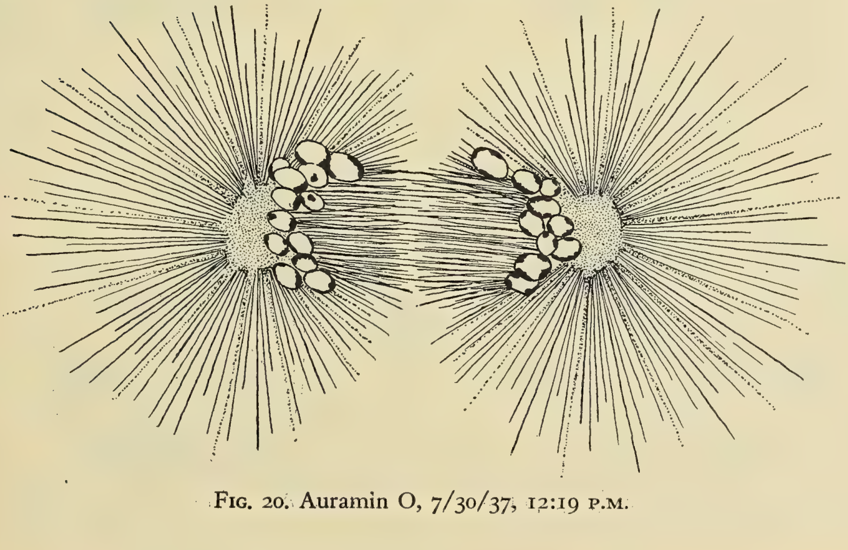


Fig. 20. Auramin O, 7/30/37, 12:19 P.M.

Many sections showed nuclei constricted as though dividing by constriction (fig. 21). A slender strand often connected the two nuclei, which varied in size (fig. 22).

There were many tripolar and tetrapolar figures, the poles of which showed either no astral radiations or greatly reduced radiations (fig. 23).

In eggs from a 1:5,000,000 solution, mitotic figures from metaphase to late anaphase were in general complete (fig. 24). The spindles were short. The centrosomes had deeply stained central bodies. The astral rays were always short.

In irradiated 1:1,200,000 cultures there were many multipolar division figures (fig. 25). These were at most only slightly astral; the great majority were anastral. In later stages there were many multipolar division figures. There were no asters or astral rays. There was little cell division. Chromatin nets, chromosomal vesicles separate or fused, granules, rods, or chromosomes, chromosomal vesicles and nuclei, or nuclear fragments, or nuclei usually of unequal size were present. There were no asters; spindles were rare, and when present were anastral. A possible nuclear constriction is shown by figure 26.

In the irradiated 1:5,000,000 culture, in general the egg nucleus had a coarsely granular net and one large and several small nucleoli, separated by a nuclear diameter from an enlarged sperm nucleus with a twisted spireme (fig. 27). Some nuclei showed an enlarged sperm nucleus just being incorporated into the egg nucleus (fig. 28). Many of the separate enlarged sperm nuclei showed two

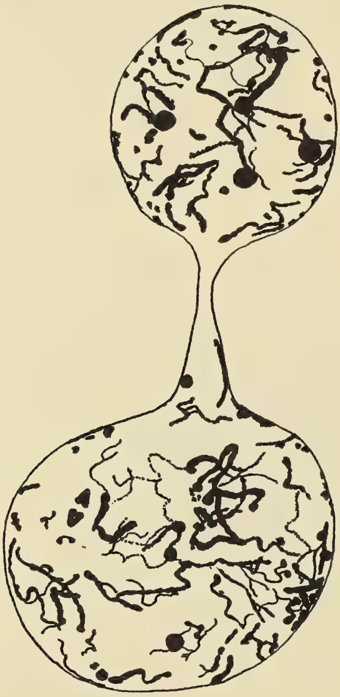


Fig. 21. Auramin O, 8/21/35, 5:40 p.m.

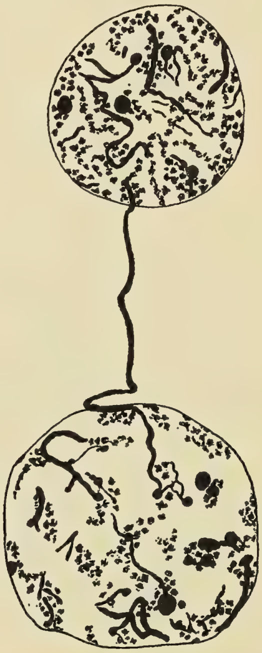


Fig. 22. Auramin O, 8/21/35, 5:40 p.m.

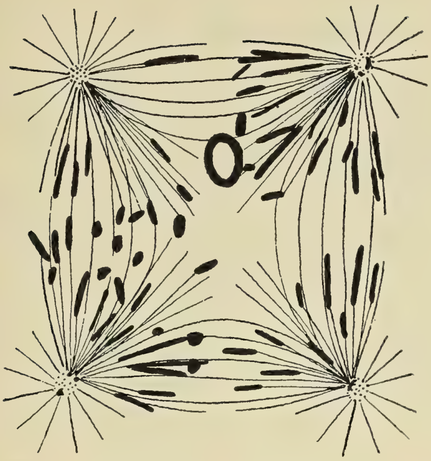


Fig. 23. Auramin O, 7/30/37, 12:19 p.m.

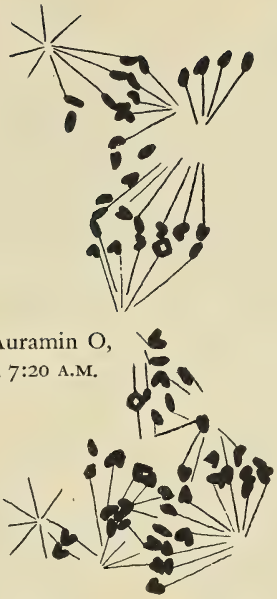


Fig. 25. Auramin O, 8/21/35, 7:20 a.m.

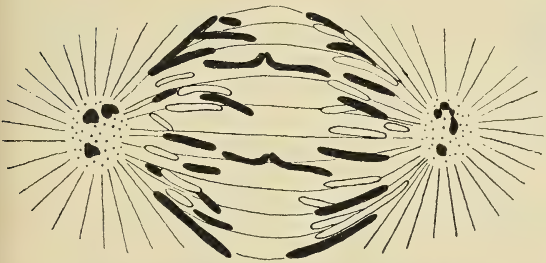


Fig. 24. Auramin O, 7/30/37, 12:19 p.m.

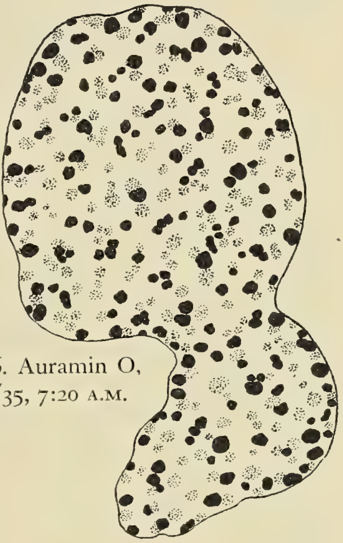
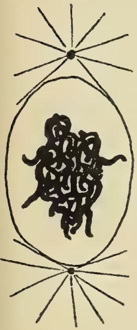


Fig. 26. Auramin O, 8/21/35, 7:20 a.m.



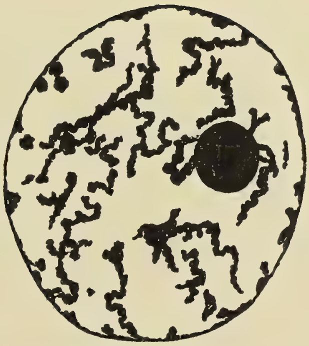


Fig. 27. Auramin O, 7/30/37, 12:29 p.m.

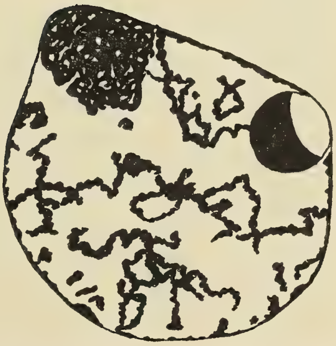


Fig. 28. Auramin O, 7/30/37, 12:29 p.m.

centers with astral rays, lying about one enlarged sperm nuclear diameter from the egg nucleus. One section showed an egg with a coarsely granular nucleus and a large nucleus constricting as though dividing.

Auramin O and neutral red. Sections of nonirradiated material show eggs with two nuclei, the egg nucleus with coarsely granular chromatin and an enlarged sperm nucleus with a twisted spireme and a clear space completely or partially surrounding the chromatin. Sometimes there were two such large sperm nuclei in the egg (fig. 29). There were many cytolizing eggs with spherules of cytoplasm attached by a stalk to the egg. The nucleus was either intact, very irregular in outline, or apparently fragmented. The partial separation of the chromatin from the nuclear wall was regarded as due to shrinkage in fixation.

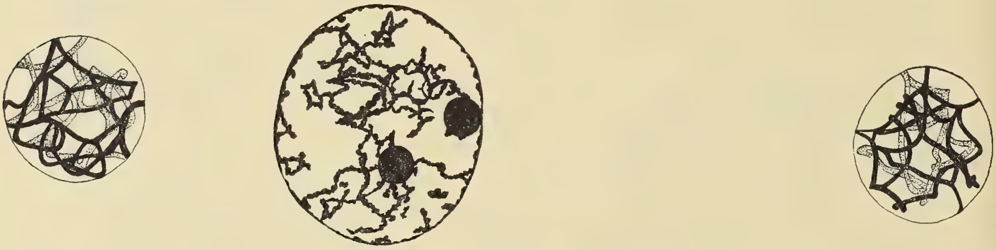


Fig. 29. Auramin O and neutral red, 8/4/37, 11:44 A.M.

In eggs irradiated in sunlight for 10 minutes there were from one to nine male nuclei, enlarged and containing a well fixed and stained loose spireme or a chromatic net extending throughout the nuclear cavity, the ends of the threads being in contact with the surrounding nuclear membrane. No shrinkage was evident in these. These nuclei, in contrast with the egg nuclei, contained no nucleoli. These eggs also showed a single large spherical nucleus, the egg nucleus, with nucleoli. Other eggs might show supernumerary sperm nuclei (e.g., five in one section), often with a good deal of shrinkage of the chromatin net. Each of these male nuclei showed diasters, one aster at each pole (fig. 30). Tripolar and tetrapolar division figures and one with five poles, prophases, metaphases, anaphases, and telophases were found. There were some groups of nuclear vesicles, each in a cone of astral rays with no rays extending outward toward the surface of the egg.

The striking characteristic of these eggs is the common occurrence of the supernumerary sperm nuclei, expanded and with a chromatic net extending from wall to wall. There are many examples of partial shrinkage (contents in contact with wall through part of its extent) and more complete shrinkage (no contact with the wall, clear space surrounding the chromatin).

Eggs irradiated 20 minutes at 29.2° C. were lobulated and cytolizing. The nuclei were fragmented, the fragments being more or less scattered. There were sometimes also one or more enlarged sperm nuclei with a chromatin net filling the nuclear cavity.

In eggs irradiated 20 minutes at a temperature of 32° C., the degree of cytol-

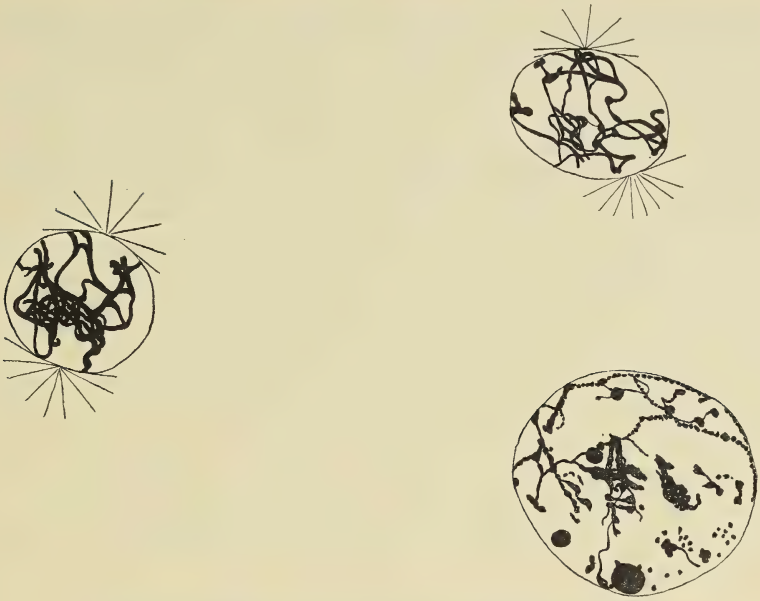


Fig. 30. Auramin O and neutral red, 8/4/37, 11:47 a.m.

ysis varied from slight to complete. The nuclei might be fragmented and the fragments scattered; they might lie in different lobes of the egg. There might be large and numerous sperm nuclei and chromosome-like rods scattered in the cytoplasm. There were instances in which a single nuclear fragment was visible and numerous rods and granules were scattered throughout the cytoplasm. There were instances in which numerous nuclear fragments appeared in the cytoplasm and no chromosome-like rods were visible. There were also instances in which the egg nucleus seemed intact but aggregations of chromatin having the appearance of disintegrating sperm nuclei were present. It seems evident that the additional 2° of temperature was accompanied by disintegration of the nuclei.

Brilliant Green

Coleman and Bell's brilliant green, dye content 95 per cent, certified, was used in concentrations ranging from 1:50,000 down to 1:1,600,000. The concentration finally selected as best suited for the experimental work was 1:800,000. Work was done with both pre-inseminated and post-inseminated *Lytechinus* eggs. The work gave results that were helpful in the interpretation of those obtained with other dyes.

A concentration of 1:50,000 is too strong, its effect being lethal in the diffuse-light culture. The cytoplasm of the eggs in these cultures was stained green within 8 minutes after the eggs were placed in the solution; during the next 20 minutes no development was evident; most of the eggs remained intact, only a few disintegrated. The eggs were beginning to cytolize 20 minutes later, or 43 minutes after insemination. In the culture irradiated and stirred, the eggs at the end of irradiation were green, intact, and coarsely granular, and showed no development; 20 minutes later they had become swollen and were beginning to break down.

In the 1:100,000 solution the cytoplasm of the eggs in the diffuse-light control stained green, no eggs divided, many showed blister cytolysis, and all died. The eggs in the culture irradiated and stirred for 20 minutes were stained very green at the close of irradiation. Blister cytolysis of these eggs began 35 minutes later and proceeded rapidly, until 30 minutes later all the eggs were dead.

The effect of both the 1:50,000 and the 1:100,000 solutions was, therefore, lethal in the nonirradiated as well as the irradiated cultures.

The cytoplasm of inseminated eggs in the 1:200,000 diffuse-light control was stained very lightly 6 minutes after being placed in the solution of brilliant green. Most of the eggs in this culture became highly irregular in form and only about 5 per cent cleaved. The cytoplasm in eggs irradiated and stirred for 20 minutes stained very blue, some eggs began to cytolize 15 minutes after the close of irradiation, and within the next 5 minutes nearly all were destroyed by blister cytolysis.

In this experiment the photodynamic effect of brilliant green became evident in the difference between the irradiated and nonirradiated cultures. None of the irradiated eggs developed, whereas about 5 per cent of the nonirradiated showed cleavage. The sublethal effect of brilliant green in the nonirradiated cultures was clear.

The cytoplasm of inseminated eggs in 1:400,000 diffuse-light cultures was very lightly stained 20 minutes after they were placed in the solution; the irradiated eggs, stirred for 20 minutes, were distinctly blue. The nonirradiated eggs gave rise to smooth abnormal 2-cell to 8-cell stages, some remaining undivided. All the irradiated eggs cytolized without normal division within 2 hours after irradiation.

The same concentration, 1:400,000, was used with three different lengths of irradiation, 10, 15, and 20 minutes. The temperatures here were 31.7°, 33.3°, and 33.9° C., respectively. Eggs in the nonirradiated diffuse-light culture stained slightly green. These eggs later became in part irregular in form, the culture finally showing 2-, 4-, and 8-cell stages as well as undivided eggs and cytolized eggs. The irradiated cultures were much alike in their behavior. In all cultures the cytoplasm stained diffusely green, the depth of color increasing with the length of irradiation. The eggs in all became slightly rough; those of the 10- and 15-minute irradiations showed considerable blister cytolysis, while those in the cultures irradiated 20 minutes showed rare blister cytolysis, but the eggs were extremely irregular and disintegrated without the formation of blisters. It seemed evident that with irradiation longer than 10 minutes the surface of the egg was so modified that blister cytolysis could not take place. As a check on the influence of high temperature during irradiation, one unstained culture was irradiated for 20 minutes in sea water, the temperature rising to 33.3° C. These eggs became indented during the course of irradiation, but smoothed up later and divided normally without cytolysis. The increased temperature without the presence of brilliant green produced a reversible reaction.

All the remaining experiments during the summers of 1937 and 1938 were on post-inseminated eggs. The eggs were irradiated for varying lengths of time at different temperatures in solutions of brilliant green of different concentrations. They were inseminated at the close of irradiation and their development followed. In 1:400,000 solutions irradiated and stirred for 10, 20, and 20 minutes at temperatures of 29.1°, 29°, and 32.7° C., the behavior of each culture was distinctly characteristic. In the nonirradiated culture the eggs were stained light green and some separated fertilization membranes, but all finally became irregular and disintegrated. The eggs irradiated for 10 minutes and cooled during irradiation were stained more deeply green and few developed fertilization membranes; more than half became irregularly blistered and finally all disintegrated.

In the cultures irradiated and stirred for 20 minutes with the temperature held at 29° C., and inseminated immediately following irradiation, no fertilization membranes were formed; many eggs became vacuolated; some blistered; most became irregular in form; all finally broke down, more than 50 per cent undergoing colorless disintegration.

In eggs in the culture irradiated and stirred for 20 minutes, not cooled, the temperature rising to 32.7° C. during irradiation, the cytoplasm became green, fertilization membranes were not formed, and many eggs became blistered within 3 minutes after the end of irradiation. Most of these eggs became irregular and showed irregular superficial clear areas. Some eggs disintegrated, and the remainder became slightly elongated with one or both ends clear. Colorless disintegration set in, the eggs finally becoming nearly spherical with a single clear area. All the eggs disintegrated without cleavage.

A series of four experiments, all with 1:800,000 solution of brilliant green, then followed. In all these experiments the time of irradiation was 10, 20, and 20 minutes, the temperature in the first two irradiations being controlled and that in the third uncontrolled. In the experiments of July 18, 1937, the eggs in the nonirradiated culture were stained slightly green, and an occasional fertilization membrane developed, but no cleavage took place. In the irradiated cultures stirred for 10 minutes and cooled to 29.4° C., the eggs stained light green. No fertilization membranes were formed, many of the eggs became vacuolated, and there was no cleavage. In the eggs irradiated and stirred for 20 minutes with the temperature held at 30° C., and inseminated at the end of irradiation, the stain was slightly deeper than in the preceding culture; no fertilization membranes were formed; there was a small amount of vacuolization, but no cleavage. In cultures irradiated and stirred for 20 minutes, the temperature not controlled and rising to 36.4° C., the eggs were inseminated at the close of irradiation. The cytoplasm in these eggs stained about the same as in the preceding culture; colorless disintegration set in about 15 minutes after insemination; the eggs swelled, became more fluid, and lost color, fully 20 per cent becoming greatly enlarged and some being blistered at each end. All the eggs showed a coarse texture. The enlarged eggs lost color completely and no divisions occurred. It seemed clear that the swelling of the eggs was a reaction to the greatly increased temperature, although it should be noted that the effect of the brilliant green in the 1:800,000 concentration was lethal even in the nonirradiated cultures. It will be noted that in the first of these experiments no fertilization membranes were formed. This is unlike the behavior in the remaining three experiments. On the morning of July 19, 1937, the eggs in the nonirradiated culture at a temperature of 28.6° C. showed above 90 per cent with fertilization membranes. Many of these eggs became irregular, and some underwent division, reaching the 2- and 4-cell stages; most did not divide; many disintegrated, showing globular cytolysis, although there were some smooth, unfertilized, undivided eggs. Eggs irradiated and stirred for 10 minutes with the temperature held at 28.5° C. were stained green, fully 90 per cent developing fertilization membranes. About 30 minutes after insemination globular cytolysis set in; later blister cytolysis appeared; there were no divisions, and fully half the eggs disintegrated following globular cytolysis. In the eggs irradiated and stirred for 20 minutes, the temperature held at 28.4° C., the eggs being inseminated at the end of irradiation, the cytoplasm was stained green, and above 90 per cent developed fertilization membranes. The eggs then became angular and globular cytolysis set in, more than 90 per cent of the eggs finally being thus involved. There was no cleavage. In the eggs irradiated and stirred for 20 minutes, the temperature not controlled and reaching 34.7° C., when the eggs were inseminated, about 50 per cent showed narrowly separated fertilization membranes. Those that had fertilization membranes were the first to blister. Within 30 minutes after insemination about half were in or entering colorless cytolysis. This increased and most of the remaining eggs became highly irregular in form, although some remained smooth.

In similar cultures made on the afternoon of the same day at a temperature of 29.4° C., the eggs stained slightly green, about half forming separated fertilization membranes. The nucleus of these eggs remained for the most part intact. There were very rare 2-cell stages, most eggs remaining spherical and only a few becoming irregular or cytolizing. A few 4-cell stages were formed later, but most of these became irregular. In the irradiated cultures, stirred for 10 minutes and cooled to 29.8° C. and then inseminated, the eggs stained green; about half formed separated fertilization membranes. The nuclei remained intact, the eggs becoming progressively irregular. Later these eggs disintegrated through colorless cytolysis, the eggs consisting of fine globules around a green core. In the eggs irradiated and stirred for 20 minutes, the temperature being kept at 29° C., the cytoplasm remained stained green, and rare fertilization membranes were formed; most of the eggs elongated and became rough; finally all disintegrated. There were no normal eggs left; all broke up into small colorless globules, sometimes surrounding a core of green. In the culture irradiated and stirred for 20 minutes without temperature control, the temperature rose to 33.7° C. The cytoplasm stained green, no fertilization membranes were formed, and the eggs became highly irregular; colorless cytolysis set in and the eggs either disintegrated or rounded up, showing a clear area against a background of solid green, and underwent no further development. The final experiment in this group was performed on the following day. Eggs in the nonirradiated 1:800,000 brilliant green culture kept in diffuse light for the most part formed well separated fertilization membranes, although a few remained only slightly separated. These eggs became angularly irregular, then gradually became lobular until about half were cytolizing. There was no cleavage. Eggs irradiated and stirred for 10 minutes at a temperature of 29.3° C. formed fertilization membranes, the cytoplasm stained green, blister cytolysis began, and within 40 minutes after insemination fully 30 per cent were lobulated or showed blister cytolysis. As time passed more than 50 per cent were in cytolysis; there was no division. In the eggs irradiated and stirred for 20 minutes with the temperature kept at 29.2° C., the cytoplasm stained green and fertilization membranes were well separated. About 20 minutes after insemination blister cytolysis appeared, increased, and reached about 50 per cent, the remaining eggs being smooth and spherical. There was no division and finally all disintegrated. In the eggs irradiated and stirred for 20 minutes with the temperature held at about 31.5° C., the cytoplasm was stained green and fertilization membranes were well formed. Blister cytolysis appeared 10 minutes after the end of irradiation; it was more prevalent than in the two preceding cultures and finally reached 90 per cent. Most of these eggs disintegrated through colorless cytolysis. There was no division.

The facts that have just been stated show that there was only rare normal early cleavage even in the nonirradiated 1:800,000 solutions of brilliant green. In the irradiated cultures, even those irradiated for only 10 minutes, there was no normal cleavage. In cultures irradiated for 20 minutes with the temperature held at 30° C. or below the destruction of the eggs was marked, and in cultures in which the temperature was permitted to rise to 33° C. and above it was even more striking.

In view of these results it seemed desirable to use even more dilute solutions of brilliant green. Therefore, some irradiations and fertilizations were made in solutions of brilliant green of 1:1,200,000 concentration. In the culture in diffuse light inseminated 13 minutes after the eggs had been placed in the solution, temperature 29.6° C., the eggs stained slightly green and excellent fertilization membranes were formed on all. Following this the eggs became slightly irregular, but later smoothed up, and 44 minutes after insemination were almost without exception in the 2-cell stage. The insemination-cleavage interval in this culture was the same as in the control. During the next 3 hours these eggs reached the 8-cell stage; the eggs in the control, however, had reached the 36-cell stage an hour and a half earlier. There was some cytolysis in the nonirradiated culture. The eggs in the irradiated culture stirred for 10 minutes and kept at a temperature of 29.4° C. were stained greener than those in the nonirradiated culture, and all developed excellent fertilization membranes. The nuclei in these eggs remained intact, there was no division, and during the succeeding 3½ hours cytolysis increased up to 50 per cent. Eggs irradiated and stirred for 20 minutes with the temperature controlled at 29.4° C. and then inseminated developed less than 100 per cent fertilization membranes and showed about 2 per cent cytolysis about 35 minutes after insemination; this percentage gradually increased, approaching 10, then 20, and finally 100 per cent. There was no division. Eggs irradiated and stirred for 20 minutes with the temperature kept at 32° C. and then inseminated developed less than 100 per cent fertilization membranes. The membranes were less well separated than in the preceding cultures. From 3 to 4 per cent globular cytolysis of both fertilized and unfertilized eggs appeared about 25 minutes after the end of irradiation. The unfertilized eggs usually showed the blister type of cytolysis. This cytolysis increased up to 10, 40, 75, and finally 100 per cent. The nuclei remained intact, and in most eggs a clear area became sharply separated from the deeply stained cytoplasm. There was no normal division.

Following this work two experiments with a 5-minute irradiation were performed. In the first of these the concentration of the solution of brilliant green was 1:1,200,000. In the nonirradiated culture in diffuse light, in which the eggs were inseminated 5 minutes after they had been placed in the solution, 80 per cent of the eggs developed fertilization membranes. Most of the eggs developed regularly into 2-, 4-, and 8-cell stages and cytolysis was very rare. In the cultures irradiated and stirred for 5 minutes with the temperature held at 30.2° C., 80 per cent of the eggs developed fertilization membranes. A slight degree of irregularity and some lobular cytolysis appeared 42 minutes after the end of irradiation. The nuclei in these eggs remained intact; there were no divisions, and cytolysis occurred in about 5 per cent of the eggs.

The next experiment was with a 1:1,600,000 solution of brilliant green. Of the eggs in the culture in diffuse light at 30.6° C., inseminated after 6 minutes exposure to the action of the dye, more than 90 per cent formed fertilization membranes. Some of these eggs divided to the 2-cell stage 54 minutes after insemination. These eggs passed on into 4- and 8-cell stages, very few remained undivided, and no cytolysis appeared. Of the eggs irradiated and stirred in the 1:1,600,000 solution of brilliant green for 5 minutes and inseminated 6 minutes after the beginning of irradiation, 80 per cent formed fertilization membranes; some showed lobular cytolysis and some lost their green color and became brownish within 45 minutes. During the next hour nuclear changes were evident in these eggs, although there were no divisions. There was rare, not more than 2 per cent, cytolysis. The nucleus remained intact in a few eggs.

Finally, it remains to consider a series of observations in which, in addition to the data on time and temperature, we have information on the intensity of irradiation. All this work was done with brilliant green in a concentration of 1:800,000. The length of irradiation in every case was 15 minutes.

The nonirradiated brilliant green control was kept in darkness throughout the experiment, which lasted about 3 hours. The eggs were inseminated 15 minutes after being placed in the solution of brilliant green. As they formed no fertilization membranes and the sea-water control showed only 50 per cent with well separated membranes, all the cultures, experimental as well as control, were reinseminated 7 minutes later. Following this insemination, the sea-water control showed 100 per cent and the dye control 50 per cent with well separated fertilization membranes. The percentage of development in the dye control was low, 20 per cent of the eggs became angular, there was no blistering, and development proceeded to the 16-cell stage. The eggs of the sea-water control during the same time had reached the 32-cell stage. The eggs in the culture irradiated with an intensity of 7747 foot-candles, at a temperature of 29.3° C., after insemination gave 25 per cent fertilization membranes. There was a gradual increase in blistering to 20 per cent; none of the eggs divided; most disintegrated. In the culture irradiated through one sheet of Whatman filter paper no. 50, giving an intensity of 4517 foot-candles at a temperature of 29.1° C., 50 per cent of the eggs formed fertilization membranes; 20 per cent became angular; blistering gradually increased until it reached 10 per cent; and the remaining eggs in the culture cleaved as far as the 16-cell stage. Eggs irradiated through three sheets of Whatman no. 50 at a temperature of 28.5° C., giving an intensity of 2363 foot-candles, developed on reinsemination 50 per cent fertilization membranes. Of these eggs 20 per cent became angular, blistering gradually increased to 5 per cent, and the remainder of the eggs cleaved from the 2- to the 16-cell stage. The eggs in another culture irradiated through a Corning glass filter no. 428, at a temperature of 29.4° C. and an intensity of 1859 foot-candles, developed reasonably well. About 25 per cent became angular for a short time, and about 5 per cent appeared to be blistering. At the end of the experiment the eggs ranged from the 2- to the 16-cell stage and about 1 per cent were blistered.

These experiments were continued with irradiation at different intensities. Twenty-five per cent of the eggs in the nonirradiated dye control kept in darkness, inseminated 25 minutes after being placed in the solution, formed fertilization membranes, but not more than 5 per cent developed to the 2-cell stage, and fewer to the 4-cell stage. Twenty-five per cent of the eggs irradiated in solutions of brilliant green at an intensity of 7532 foot-candles and a temperature of 31.2° C., inseminated 10 minutes after the end of irradiation, formed membranes; 10 per cent blistered; none divided. Twenty-five per cent of the eggs irradiated in the solutions through three sheets of Whatman filter paper no. 50, at an intensity of 2147 foot-candles and a temperature of 30.4° C., inseminated 10 minutes after the end of irradiation, formed fertilization membranes; none blistered; cleavage proceeded somewhat irregularly to the 4-cell stage. Twenty-five per cent of the eggs in the culture irradiated through a Corning glass filter no. 428 at an intensity of 1808 foot-candles and a temperature of 30.7° C., inseminated 10 minutes after the end of irradiation, showed fertilization membranes; there was blistering on 2 per cent of the eggs; none of them cleaved. Finally, 25 per cent of the eggs irradiated in the solutions through a sheet of red glass at an intensity of 226 foot-candles and a temperature reaching 30.9° C. during irradiation, inseminated 10 minutes after the end of irradiation, showed fertilization membranes. None of the eggs in this culture blistered; somewhat rare 2-cell and 4-cell stages were formed.

In 1939, brilliant green cultures, 1:800,000, were prepared for photographic purposes. The following table gives the results.

Summary of Results of Irradiation of Eggs in Solutions of Brilliant Green 1:800,000

Date	Culture no.	Temperature (°C.)	Time (min.)	Intensity of sunlight (foot-candles)
7/21/39	(1)	29.2	0	Dark
	(2)	31.0	20	8633

Results. (1) 5 minutes after insemination a few fertilization membranes; 59 minutes and 60 minutes after insemination cytolysis 10 per cent plus.

(2) 5 minutes after insemination beginning to form lobules; photographed, plate 3, figures 3 to 6, and plate 4, figures 1 to 5; 11, 19, 22, 24, 38, 44, and 51 minutes after insemination, showing lobular cytolysis; plate 4, figure 4 shows the progression of colorless cytolysis, and plate 4, figure 5, 97 minutes after insemination, shows completed colorless cytolysis.

Results of the Study of Sections of Eggs from Brilliant Green Cultures

In both nonirradiated 1:400,000 and 1:800,000 solutions of brilliant green there was slight lagging of the chromosomes during division. In the 1:800,000 solution there were many eggs in which there was no lagging.

In the eggs irradiated 10 minutes in 1:400,000 solution, there was no normal mitotic division. The nucleus seemed to fragment. There was never anything in the nature of a normal mitotic figure. In eggs irradiated for 10 minutes in 1:800,000 solution no division occurred.

In eggs irradiated for 10 minutes in 1:1,200,000 solution, the nucleus remained intact. Several male nuclei entered the egg, but no development took place. In eggs irradiated for 5 minutes in 1:1,600,000 solution, division was rare but cytolysis was also rare.

Figures 31 and 32 were drawn from eggs irradiated 10 minutes in a 1:1,200,000

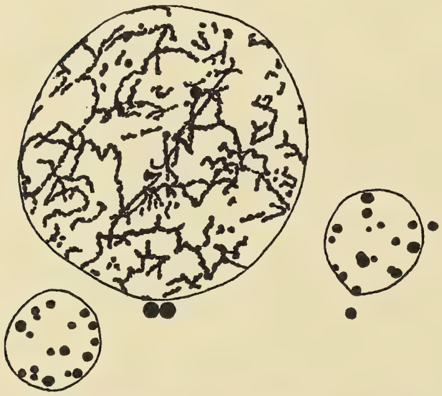


Fig. 31. Brilliant green, 7/21/37, 1:40 P.M.

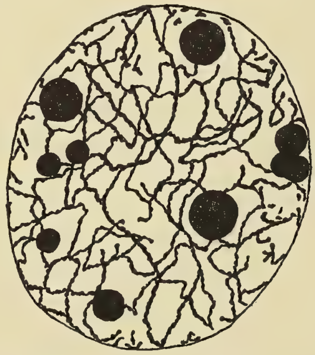


Fig. 32. Brilliant green, 7/21/37, 1:40 P.M.

solution of brilliant green. In figure 31 supernumerary sperm nuclei are shown. In both of these the chromatin net has been reduced to prochromosome-like structures, eighteen in one and seventeen in the other. In the other figure (fig. 32) there are nine large nucleoli. This condition is common.

Basic Fuchsin

National Aniline Company's basic fuchsin, certified, dye content 82 per cent, was used in all the experiments described below.

The first experiments were performed with basic fuchsin in 1:50,000 solution in sea water. Eggs were placed in solutions of basic fuchsin 5 minutes after insemination and kept in diffuse light. The eggs remained spherical and had a normal appearance. Division into 2 cells began 38 minutes after insemination. The eggs divided progressively into 2-cell and 4-cell stages, a few dividing to 8-cell stages 1½ hours after insemination. After this many of the 2-cell and 4-cell stages began to blister cytolize, and within 2½ hours all stages were disintegrating. Eggs which were transferred 5 minutes after insemination to 1:50,000 basic fuchsin, and irradiated and stirred for 10 minutes, stained yellowish pink during the irradiation and very quickly became irregular in form. In general these eggs became spherical again within 20 minutes. An occasional egg had divided to 2 cells 47 minutes after insemination, but most of the eggs in the culture became irregular in form, and some showed blister cytolysis. Many eggs had divided to 2 very irregular blastomeres 20 minutes later; irregularity and cytolysis were evident in the undivided eggs. Still later, undivided eggs, 2-cell stages, rare 4-cell stages, and about 50 per cent blister cytolysis marked these cultures. The final result, 4 hours after insemination, was the complete disintegration of all stages. The result of this experiment makes it clear that 1:50,000 solutions of basic fuchsin were too strong.

Basic fuchsin was then used in 1:100,000 solutions. Inseminated eggs were transferred 5 minutes after insemination to 1:100,000 solution at a temperature of 31.2° C. The eggs in these cultures, 4 minutes later, were unstained and had retained a fine, spherical form. These eggs, like the eggs in the sea-water control, passed into the 2-cell stage 37 minutes after insemination. Their progressive cleavage into 2-, 4-, 8-, 12-, and 16-cell stages was normal and regular, paralleling that of the control. Their development into blastulae was also normal, but on the following morning all were dead in the gastrula stage. Other eggs were transferred 5 minutes after insemination to a 1:100,000 solution, in which they were irradiated and stirred for 10 minutes, the temperature rising during irradiation to 34.6° C. This temperature is so high that one should expect heat cytolysis as a result. Immediately after the completion of irradiation the eggs were spherical and slightly yellow in color. They had become slightly irregular in form 17 minutes later, and then began to cytolize. After another 25 minutes most of the eggs in the culture were blister cytolizing, only a few eggs completing a poor first division. None of the eggs that had received this treatment gave rise to a normal stage; all cytolized without further division. The result of these experiments indicates that eggs develop well in nonirradiated 1:100,000 solutions of basic fuchsin kept in diffuse light. The results on the irradiated cultures are not conclusive because of the high temperature to which the cultures were subjected during irradiation.

In the succeeding experiments 1:200,000 solutions of basic fuchsin were used. In these experiments uninseminated eggs were transferred to solutions kept in diffuse light at 29° C. and inseminated 12 minutes later. These eggs formed excellent fertilization membranes and showed very slight staining of small granules in the cytoplasm, the larger granules not being stained. The eggs in this culture began division to the 2-cell stage 36 minutes after insemination. They divided regularly and normally to 2- and 4-cell stages and gave rise to normal blastulae, gastrulae, and prism larvae. Uninseminated eggs of the same lot were transferred to 1:200,000 solution of basic fuchsin, irradiated and stirred for 10 minutes with the temperature held at 29.3° C., and inseminated 2 minutes after the end of irradiation. These eggs formed excellent fertilization membranes, retained their spherical form well, underwent normal cleavage, and on the following morning were in either the early gastrula or the prism-larva stage. Uninseminated eggs transferred to 1:200,000 solution of basic fuchsin, and irradiated and stirred for 20 minutes with the temperature during irradiation reaching 28.8° C., were inseminated 2 minutes after the end of irradiation. These eggs formed excellent fertilization membranes, showed some irregularity of form, and began to constrict for the 2-cell stage 40 minutes after insemination. They cleaved regularly and developed through normal blastula and gastrula stages. The remaining culture of this series contained uninseminated eggs which were transferred to the 1:200,000 solution of basic fuchsin and irradiated and stirred for 20 minutes at 32° C. They were inseminated 2 minutes after the end of irradiation. They developed excellent fertilization membranes. The eggs in the culture became rather misshapen, more so than those in any other culture of this series. They began their constriction for the first division 39 minutes after insemination. These eggs showed considerable polyspermy and formed rather bad 2- and 4-cell stages. Some of them continued their development and on the following morning had reached the gastrula and prism-larva stage. All these cultures, the sea-water control as well as the experimental cultures just described, showed similar active larvae. Except for the last irradiated culture, none showed anything like a differential death rate. The most obvious comment that can be made is that treatment slowed the later development.

Following these experiments, another series of experiments with 1:100,000 solutions of basic fuchsin was performed. Uninseminated eggs were transferred to solution of basic fuchsin and inseminated in this solution 11 minutes later at a temperature of 29.2° C. These eggs formed excellent fertilization membranes, and most of the eggs remained beautifully spherical. They reached the 2-cell stage in the same time as those in the sea-water control, 42 minutes after insemination. Like the eggs in the control, they passed regularly to the 4-, 8-, 12-, and 16-cell stages. They were followed no farther. Uninseminated eggs transferred to 1:100,000 solution of basic fuchsin, irradiated and stirred for 10 minutes with the temperature held at 29.2° C., and inseminated 1 minute after the end of irradiation, formed no separated fertilization membranes. Many of the eggs in this culture were angularly indented, with the membrane raised over the indentation. This condition persisted for some time. A few of these eggs showed constriction and division to the 2-cell stage, as well as some polyspermic 4-cell stages, 46 minutes after insemination. Most of the eggs had reached the 4-cell stage 15 minutes later. These eggs continued their cleavage to the 16-cell stage. They were followed no farther. Many cleavages were irregular. Uninseminated eggs transferred to the 1:100,000 solution of basic fuchsin, irradiated and stirred for 20 minutes with the temperature held at 29.4° C., and inseminated 1 minute after the end of irradiation, formed no separated fertilization membranes. These eggs became very much indented 15 minutes after insemination, but none cytolized. Slightly later they showed close fertilization membranes, some blister cytolysis, and considerable wrinkling. They showed no cleavage, but exhibited greatly increased cytolysis 20 minutes later, or 38 minutes after insemination. They began to cleave to irregular and partial 2-cell stages and showed much cytolysis of undivided eggs 48 minutes after insemination. Some of the eggs in the culture formed regular 4-, 8-, 12-, and 16-cell stages. The majority of eggs showed irregular cleavage. The final culture in this series initially contained uninseminated eggs which were transferred to 1:100,000 solution of basic fuchsin, irradiated and stirred for 20 minutes with the temperature held at 32° C. (finally 31.6° C.), and inseminated 1 minute after the end of irradiation. These eggs at first were somewhat brownish and showed no separated fertilization membranes. Some of them showed blister cytolysis 15 minutes after insemination. Slightly later, closely fitting fertilization membranes could be seen on the eggs. Blister cytolysis increased greatly. Many polyspermic nuclear changes became evident about 40 minutes after insemination. There was some irregular and partial division of these eggs to incomplete 2-cell stages 48 minutes after insemination. Some of these eggs completed division to the 2-, 4-, 8-, 12-, and 16-cell stage. Irregularity in division was greater than in any of the other cultures, this being the only culture in which the blastomeres separated from each other.

Results of the Study of Sections of Eggs from Basic Fuchsin Cultures

Miss Margaret Evans studied sections of two lots of *Lytechinus* eggs that had been irradiated in basic fuchsin. The first lot consisted of eggs that had been irradiated for 20 minutes in 1:200,000 solution of basic fuchsin, during which they were cooled and then inseminated. Fifty-eight per cent of these eggs divided normally and 42 per cent abnormally. Twenty-two per cent showed pycnosis of chromosomes and 5 per cent fragmentation of chromosomes. Thirty per cent showed multipolar spindles. Forty-eight per cent showed condensations in the cytoplasm, involving both the hyaloplasm and the cytoplasmic granules, and very marked condensations on the surface of the egg. Cytolysis did not occur.

The second lot consisted of eggs that had been irradiated in 1:100,000 solution of basic fuchsin for 20 minutes at a temperature of 32° C. and then inseminated. About 33 per cent of these eggs were normal and 67 per cent were abnormal. Twenty-four per cent showed pycnosis of chromosomes and 3 per cent fragmentation of chromosomes. Thirty-four per cent showed multipolar spindles and 5 per cent showed 2 separate spindles. Condensations appeared in about 26 per cent of these eggs. Cytolysis with nuclear fragmentation occurred in 10 per cent of the eggs and blister cytolysis in 14 per cent.

Polyspermy seemed increased in the stronger solution. Not only were there more multipolar spindles, but there were also instances in which the supernumerary sperm nuclei were not in any way connected with the zygote nucleus.

Rosolic Acid

The Coleman and Bell Company's rosolic acid, pH range 6.9 to 8.0, was used in these experiments. It was used in concentrations varying from 1 cubic centimeter saturated solution of rosolic acid in distilled water to 250 cubic centimeters sea water, up to a saturated solution of rosolic acid in sea water.

In the first experiment, eggs were transferred 10 minutes after insemination to a mixture of 1 cubic centimeter saturated solution of rosolic acid in distilled water to 250 cubic centimeters of sea water, and the culture kept in diffuse light. The development of eggs in this culture was identical with the development of eggs of the same insemination transferred 10 minutes after insemination to a similar solution of dye and irradiated for 20 minutes. There was no sunshine, the day being somewhat cloudy. The eggs of both these cultures were entering the 2-cell stage 40 minutes after insemination. The dye had no effect either in diffuse light or in irradiated cultures.

Similarly, cultures were made with 4 cubic centimeters saturated solution of rosolic acid in 250 cubic centimeters of sea water. The development in the diffuse-light cultures and in the cultures irradiated for 20 minutes (cloudy sky, no sunshine) was the same. The eggs of both cultures were entering the 2-cell stage 49 minutes after insemination. Rosolic acid had no effect either in diffuse light or in the irradiated culture.

The remaining experiments were done with saturated solution of rosolic acid in sea water. Eggs were transferred 6 minutes after insemination to saturated solution of rosolic acid in sea water and kept in diffuse light. These eggs were entering the 2-cell stage 39 minutes after insemination. They continued to cleave, some of them abnormally and irregularly, so that the culture contained, 1¾ hours after insemination, stages from 4 to 16 cells. Eggs transferred 6 minutes after insemination to a similar solution of rosolic acid were irradiated and stirred for 20 minutes. These eggs did not enter the 2-cell stage at the same time as the sea-water control and the diffuse-light culture. None divided regularly, but a few went through early irregular divisions. There were no normal divisions, and some eggs were cytolizing 1¾ hours after insemination. The culture showed no development.

Two additional cultures were prepared in the same way. In the diffuse-light culture made by transferring eggs 5 minutes after insemination to saturated solution of rosolic acid in sea water, cleavage proceeded regularly in the same tempo as in the sea-water control to a late cleavage stage with a large cleavage cavity. The irradiated culture was made by transferring eggs of the same insemination 5 minutes after insemination to saturated solution of rosolic acid in sea water. These were then irradiated and stirred for 20 minutes. Cleavage, although in the same tempo as that of the control, was irregular from the beginning. It also was followed to a late cleavage stage with a large cleavage cavity.

PYRONIN

In these experiments the National Aniline and Chemical Company's pyronin, certified by the Commission on Standardization of Biological Stains, was used in concentrations varying from 1:25,000 to 1:150,000. The greater part of the work was done with sea-water solutions of the latter concentration.

The cytoplasm in *Lytechinus* eggs transferred, 5 minutes after insemination, to 1:25,000 solutions of pyronin in sea water and kept in diffuse light was at once stained distinctly pink by this dye. Most of the eggs were blister cytolized 30 minutes after insemination. No eggs divided. Inseminated eggs transferred to 1:25,000 solution of pyronin in sea water 5 minutes after insemination were irradiated and stirred for 20 minutes. These eggs were redder than the eggs of the nonirradiated culture, and a few were cytolizing 15 minutes after the end of irradiation. None of these eggs divided; all were dead 90 minutes after transfer to the solution of pyronin. None blister cytolized. It was evident that 1:25,000 solution of dye was too strong, since there was no division even in the nonirradiated culture. It was evident, further, that 20 minutes irradiation in a solution of this strength killed the eggs before blister cytolysis could take place.

Simultaneously a study was made of the effect of 1:50,000 solutions of pyronin in sea water on these eggs. *Lytechinus* eggs of the same insemination were transferred 5 minutes after insemination to 1:50,000 solution of pyronin in sea water and kept in diffuse light. These eggs were distinctly less pink than those of the 1:25,000 diffuse-light culture. None of these eggs divided, and blister cytolysis had killed most of them 90 minutes after insemination. Eggs transferred to 1:50,000 solution of pyronin in sea water 5 minutes after insemination and irradiated and stirred for 20 minutes were redder than those in the diffuse-light culture. Some eggs began to cytolize within 15 minutes after the end of irradiation. Blister cytolysis gradually increased until, 90 minutes after insemination, most of the eggs were blister cytolized and none had divided. At the conclusion of this experiment it was clear that this solution, like the 1:25,000 solution, was too strong even in the nonirradiated cultures.

Experiments were therefore performed in which more dilute solutions of pyronin were used. In the first of these experiments, *Lytechinus* eggs were transferred 5 minutes after insemination to 1:100,000 solution of pyronin in sea water and kept in diffuse light. The cytoplasm of these eggs was stained light pink 3 minutes after transfer to the dye. Many of the eggs were irregular in form, and none were dividing 39 minutes after insemination. Some had entered the 2-cell stage 8 minutes later. Not more than 10 per cent were in the 2-cell stage 20 minutes later, or 67 minutes after insemination. Those dividing lost their color; those retaining their color did not divide. Eggs transferred to 1:100,000 solution of pyronin in sea water 5 minutes after insemination were irradiated and stirred for 20 minutes. At the close of irradiation the cytoplasm of these eggs was stained deep pink. The plasma membrane was slightly irregular in many. Many eggs had become irregular in form 6 minutes after the close of irradiation, and most were blister cytolizing 10 minutes after the close of irradiation. All these eggs had blister cytolized 23 minutes after the end of irradiation. Eggs of the sea-water control divided normally. It thus became evident that the 1:100,000 solution of pyronin was too strong, and in the next experiment a more dilute solution was used.

Lytechinus eggs were transferred to 1:150,000 solution of pyronin in sea water 5 minutes after insemination and kept in diffuse light. In this culture no staining of the eggs was evident 13 minutes after the transfer to the solution of pyronin. A few eggs were entering the 2-cell stage and some undivided eggs had an irregular outline 39 minutes after insemination. All the eggs of this culture were in either the 2-cell or the 4-cell stage 1 hour after insemination. These eggs continued their cleavage regularly. Eggs of the same insemination were transferred to 1:150,000 solution of pyronin in sea water 5 minutes after insemination and irradiated and stirred for 20 minutes. These eggs were stained pink at the end of irradiation. They had lost their pink stain 15 minutes later; some of the eggs had an irregular outline and some were blister cytolized. None of these eggs had divided 1 hour after insemination. None had divided and cytolysis had become general 2 hours later, or 3 hours after insemination. It seemed evident, then, from this experiment, that the concentration 1:150,000 was about that at which normal cleavage in diffuse-light cultures might take place, and in which irradiation with sunlight produced a photodynamic effect.

The next four sets of experiments that were performed with 1:150,000 solution of pyronin in sea water, at approximately the same temperature, gave results that were similar to each other. Inseminated eggs were transferred 5 minutes after insemination to diffuse light at a temperature of 29° C. These eggs 8 minutes after the transfer were unstained, had sharp outlines, and showed radiations in the cytosome around the nucleus. The nucleus in these eggs was stained slightly pink 22 minutes after being placed in the solution of dye. These eggs divided to give excellent regular 2-cell stages, division being somewhat slower than in the sea-water control. They began to enter the 4-cell stage 64 minutes after insemination, at which time their color had become normal. Their cleavage then became irregular in rhythm; eggs in the 2- to the 16-cell stage were present in this culture 2 hours and 20 minutes after insemination. Eggs of the same insemination, transferred to 1:150,000 solution of pyronin in sea water 5 minutes after insemination, were irradiated and stirred for 10 minutes. The temperature then stood at 31.8° C. These eggs were stained pink and had a sharp outline at the close of irradiation. They had lost their stain and a little cytolysis was in evidence 41 minutes after insemination, the nucleus being intact. The eggs were still in this condition 1 hour after insemination. No division had occurred and about 50 per cent had blister cytolized 2 hours and 20 minutes after insemination. Another portion of these eggs was transferred to 1:150,000 solution of pyronin in sea water 5 minutes after insemination and irradiated and stirred for 20 minutes. At the close of irradiation the temperature stood at 34.1° C. These eggs 28 minutes after insemination (3 minutes after the end of irradiation) were pinker than those of the 10-minute irradiation. The unfertilized eggs in the culture were not stained and there was a very small amount of blister cytolysis. Blister cytolysis increased, about a third of the eggs cytolizing during the next 15 minutes. None of the eggs had divided and about half had cytolized 55 minutes after insemination. Cytolysis increased slightly, and there was no cleavage during the time that the sea-water control was followed to late cleavage.

Inseminated eggs were transferred 8 minutes after insemination to the solution of pyronin and kept in diffuse light at a temperature of 27.6° C. During the first 20 minutes after transfer to pyronin the eggs remained unstained and became irregular. Eggs in the sea-water control began to divide 40 minutes after insemination. A few eggs in the diffuse-light culture had divided 3 minutes later. These eggs were followed as far as the 4-cell stage, which they had reached in tempo with the sea-water control 68 minutes after insemination. A few undivided eggs cytolized during this time. Eggs transferred to the solution of pyronin 8 minutes after insemination were irradiated and stirred for 15 minutes. At the end of this time the temperature stood at 32.4° C., a rise of nearly 5° during irradiation. At the close of irradiation these eggs had become pink in color and irregular in outline. Many eggs had begun to cytolize 40 minutes after insemination. From this time on the eggs began to lose their stain, while cytolysis increased. Most of the eggs showed no stain, 10 per cent were cytolized, and the remainder were spherical and undivided 65 minutes after insemination. No divisions were observed in these eggs up to the time when the cleavage in the sea-water control reached the completed 4-cell stage. Eggs of the same insemination were transferred to solution of pyronin in sea water 8 minutes after insemination and irradiated and stirred for 20 minutes. The temperature at the close of irradiation was 32.4° C. At this time the eggs were stained pink and were irregular in form. Cytolysis was more common in this culture than in the preceding culture, having reached 30 per cent 25 minutes after the close of irradiation. No division occurred in these eggs, and cytolysis gradually increased until it had reached 40 per cent 73 minutes after insemination.

The next experiments were almost precisely similar. The temperature of the diffuse-light culture was 28.6° C. The eggs in this culture proceeded through a rather variable cleavage, only undivided eggs and 2-cell and 4-cell stages, which looked normal, being in the culture when the eggs of the sea-water control had reached a late cleavage stage. Eggs transferred to solution of pyronin in sea water 5 minutes after insemination were irradiated and stirred for 15 minutes. The temperature of this culture had then reached 32.6° C. The eggs were stained and were slightly irregular in form at the close of irradiation. None of these eggs had divided and 25 per cent had cytolized 42 minutes after insemination. Cytolysis had increased to 50 per cent 1 hour and 25 minutes after insemination, and to 98 per cent, with 2 per cent remaining undivided, 3 hours after insemination. Essentially the same statements may be made concerning the eggs that were irradiated and stirred in solution of pyronin in sea water for 20 minutes. At the close of irradiation the temperature had reached 33.8° C. The eggs were stained and were slightly irregular in form. One hour after insemination 98 per cent of these eggs were cytolized, and 2 per cent remained smooth and spherical. When observations were discontinued, more than 99 per cent had cytolized. In the next experiment, though the amount of cytolysis was high, it did not reach the level described above. Eggs were transferred 5 minutes after insemination to solution of pyronin in sea water and kept in diffuse light at a temperature of 28.4° C. These eggs remained unstained and only a few became irregular in form. They began to enter the 2-cell stage, in tempo with those of the sea-water control, 40 minutes after insemination. They continued their cleavage regularly and were followed to the 8-cell stage. Inseminated eggs transferred to solution of pyronin in sea water 5 minutes after insemination were irradiated and stirred for 15 minutes. At the end of irradiation the temperature had reached 32.8° C. The eggs were then pink, and many were irregular in outline. Within the next 2 minutes some of the eggs became brownish, and these almost immediately became irregular in outline. A coarsely granular cytolysis appeared in many of the eggs in this culture 10 minutes after the end of irradiation. Cytolysis increased rapidly until, 1 hour and 20 minutes after insemination, it had reached more than 50 per cent. No cleavage occurred in the culture. The same description, no cleavage and more than 50 per cent cytolysis, may be given for the eggs that were transferred to solution of pyronin in sea water 5 minutes after insemination and irradiated and stirred for 20 minutes. The temperature in this culture reached 34.6° C. It is evident that these four sets of experiments have in common excessive temperature as a factor to be considered. It will be noted that there was no cleavage in any of the irradiated cultures and that the percentage of cytolysis was high. In the diffuse-light controls of each of these experiments, cleavage was regular in form, although the tempo of later cleavage seems to have been somewhat disturbed. It seemed evident that the high degree of cytolysis occurring in the irradiated cultures is to be correlated with excessive temperature. Further experiments with controlled temperatures therefore seemed desirable. The next three sets of experiments gave results confirming this opinion. This work also was done with 1:150,000 solution of pyronin in sea water.

Inseminated *Lytechinus* eggs were transferred to solution of pyronin in sea water 5 minutes after insemination and kept in diffuse light at a temperature of 27° C. Granules in the eggs were stained faintly pink. Eggs were entering the 2-cell stage 44 minutes after insemination, in tempo with the control. Cleavage was followed in both to the 16-cell stage, which was reached 2 hours after insemination. Eggs were transferred 5 minutes after insemination to solution of pyronin in sea water and irradiated and stirred for 15 minutes. The culture was cooled during this process, and at the end of irradiation its temperature was 24.8° C. During irradiation the sky was overcast but bright. No direct sunlight was evident. The eggs of this culture were slightly irregular in outline at the close of irradiation. About 10 per cent were in the 2-cell stage 55 minutes after insemination, and most had divided or were dividing 5 minutes later. The cleavage in these eggs was followed until those in the control and in the diffuse-light culture had reached the 16-cell stage. The eggs irradiated for 15 minutes were then in the 4- and 8-cell stages. Eggs transferred to solution of pyronin in sea water 5 minutes after insemination were irradiated and stirred for 20 minutes. The temperature of this culture was controlled at 24.6° C. Its history is the same as that of the 15-minute irradiation just described. Cleavage was followed until the eggs had reached the 4-cell and 8-cell stages. It is clear that the only effect of irradiation in these experiments was the slowing of development in the irradiated cultures. It is possible that the reduction of temperature from 27° to 24.8° and 24.6° C., respectively, was responsible. In the next experiment the temperature was not reduced so far. Eggs were transferred 5 minutes after insemination to solution of pyronin in sea water and kept in diffuse light at a temperature of 28.1° C. The cytoplasm of the eggs was not stained, although faintly pink granules could be seen 10 minutes after the eggs had been placed in the solution of pyronin. The eggs were passing into the 2-cell stage 42 minutes after insemination. Most of these eggs were in the 2-cell stage when all those of the sea-water control were in the 2-cell stage, 49 minutes after insemination. Cleavage was regular and slightly slower than in the control. When the eggs of the control 5 hours later had developed to rotating blastulae, those of the diffuse-light culture were young but nonrotating blastulae. The culture also contained some 2- to 8-cell stages and irregularly cleaving eggs. On the day following, the control contained fine plutei, whereas the diffuse-light culture contained no plutei, a few rotating blastulae, and no gastrulae; most of the embryos were in the form of disintegrating blastulae. Eggs transferred to solution of pyronin in sea water 5 minutes after insemination and irradiated and stirred for 15 minutes were held at a temperature of 28.1° C. At the close of irradiation the eggs were very pink and slightly irregular in form. During the next 15 minutes many eggs became very irregular, and some cytolizing eggs had become brownish instead of pink. About this time some of the eggs became indented. About 50 per cent of the eggs had become highly irregular in form, with the nucleus still intact, 50 minutes after insemination. Most of these eggs had become spherical, with the nucleus still intact, 90 minutes after insemination. At this time very rare 2-cell stages were evident. Five hours after irradiation the culture contained undivided eggs and cytolized eggs in about equal numbers. On the following morning some of the eggs were still intact, most of them were disintegrating, and there were no active stages. The development of inseminated eggs irradiated and stirred for 20 minutes was essentially the same. The temperature of this culture at the close of irradiation was 29.4° C. The eggs were pinker than those of the 15-minute irradiation. Many of the eggs had become irregular (indented) 15 minutes after irradiation. About 50 per cent were indented and were becoming brown 20 minutes after the close of irradiation. Cytolysis appeared in this culture 54 minutes after insemination and increased slowly in amount until it reached 5 per cent 1 hour and 40 minutes after insemination. After 5 hours the culture contained undivided and cytolized eggs, the former predominating. On the following morning the culture contained many intact 1-cell stages, and the remainder were disintegrating like those of the 15-minute culture. The cultures just described were developing at favorable temperatures, the same as those of the sea water in the Tortugas region. It was evident that development in the diffuse-light culture was slightly less good than in the sea water. It was further evident that the photodynamic action of the dye had retarded development and had produced cytolysis and disintegration in the irradiated cultures. It was still further evident that the effect of irradiation at these normal temperatures was not so destructive as at the higher temperatures (32° to 34° C.) and that development was not so slow as at lower temperatures (24.6° to 24.8° C.).

The next experiment was performed at normal temperature and with shorter irradiation. During this experiment the sky was overcast and little sunlight was visible. The mixed eggs of three females were used, as no single individual with a sufficient quantity of eggs was available. Eggs were transferred 6 minutes after insemination to solution of pyronin in sea water and kept in diffuse light. The temperature of the solution was 28.6° C. The eggs remained unstained. Many became irregular, although some remained spherical. Some began to divide 37 minutes after insemination, and 48 minutes after insemination 25 per cent were in the 2-cell stage, all the eggs of the sea-water control being in the 2-cell stage at this time. When the eggs of the control showed 4-cell and 8-cell stages, 90 minutes after insemination, a culture in 1:150,000 solution of pyronin in sea water contained regular 1-, 2-, 4-, and 8-cell and irregular 1-cell stages. Three hours after insemination this culture contained stages from the 1-cell to the 64-cell and cytolized eggs, while all of the eggs of the sea-water control were in regular late cleavage stages, about 128 cells. Eggs were transferred 6 minutes after insemination to solution of pyronin in sea water and irradiated and stirred for 5 minutes. At the close of irradiation the controlled temperature was 28.8° C. These eggs were stained pink and were slightly irregular in form at the end of irradiation. Twenty per cent were in the 2-cell stage 49 minutes after insemination. Many of the remaining eggs were irregular in form. Among the eggs of this mixed culture those of one female were distinctly smaller. These eggs were unstained and were among the first to divide. Cleavage continued in this culture irregularly and more slowly than in the control and at about the same rate as the eggs of the diffuse-light culture. They had reached the same stage of development as these 3 hours after insemination. Eggs transferred to solution of pyronin in sea water 6 minutes after insemination were irradiated and stirred for 10 minutes. At the close of irradiation the temperature was 28.5° C. The eggs were stained pink and their form was irregular. Many of them became indented. Most of the eggs of this culture were stained 30 minutes after insemination, although some small ones were spherical and unstained. These small eggs behaved like those of the 5-minute culture, and 42 minutes after insemination they were constricting for the first division. The culture contained, 7 minutes later, 20 per cent of eggs in the 2-cell stage, all these having come from the division of the small unstained eggs. These eggs divided regularly to 4-cell stages and continued their division regularly to the 64-cell stage. The other eggs of the mixed culture showed variable behavior, and 3 hours after insemination provided the 1-, 2-, and 3-cell and cytolized eggs that were found in the culture. Similarly, eggs were transferred 6 minutes after insemination to solution of pyronin in sea water and irradiated and stirred for 15 minutes. At the end of this time the controlled temperature was 28.1° C. These eggs were stained pinker than those of any of the other irradiations, although the small spherical eggs in which regular nuclear division could be followed were unstained and distinctly recognizable. In this culture 42 minutes after insemination most of the stained eggs were irregular, and the small unstained eggs were constricting for the first division. These eggs furnished the 20 per cent of eggs in the 2-cell stage present in this culture 50 minutes after insemination. The culture contained, 3 hours after insemination, normal 64-cell stages, undivided eggs, and cytolized eggs.

The results of this experiment indicate clearly the fact that the eggs of different individuals vary in their susceptibility to irradiation. The eggs of one individual were recognizable because of their smaller size. The small eggs in the mixed culture not only did not stain, but were not affected by irradiation. Because of this individual behavior of the eggs of one female, it is impossible to conclude from this experiment that the photodynamic effect of pyronin had increased proportionately with the duration of irradiation. None of the treatments given were sufficient to affect the eggs of this one female.

The final experiments were performed with eggs that were not inseminated until after their treatment with 1:150,000 solution of pyronin in sea water. Uninseminated eggs were transferred to the solution of dye and kept in diffuse light at a temperature of 29.4° C. They were inseminated in this solution 20 minutes later. The eggs were not stained and formed a very tight fertilization membrane, the surface of the eggs and the plasma membrane being wrinkled 25 minutes after insemination. The eggs remained in this condition, while those of the sea-water control were reaching the 4-cell stage. Eggs were transferred to solution of pyronin in sea water and irradiated and stirred for 20 minutes. The temperature, which was controlled, then stood at 29.2° C. These eggs were inseminated at the end of irradiation. They were stained slightly pink, and the tight fertilization membrane was evident 25 minutes later. The surface of the eggs and the plasma membrane were wrinkled. Blistering appeared in the culture 30 minutes after insemination. These eggs did not divide and had the same appearance as those of the diffuse-light culture. Eggs were transferred to solution of pyronin in sea water, irradiated and stirred for 20 minutes, and inseminated at the close of irradiation. The culture was not cooled during irradiation and the temperature at the time of insemination stood at 35.5° C. The eggs were stained pink; no fertilization membrane was separated. Most of the eggs had blistered 25 minutes after insemination. All the fertilized eggs were cytolizing irregularly a half-hour later. The results of this experiment were in substantial agreement with those previously given in that eggs in the diffuse-light culture were not stained, whereas those irradiated in sunlight were stained readily, the intensity of staining depending on the duration of irradiation. The results of this experiment also agreed with the results described above as to the effect of excessive temperature. It should be noted that the high degree of cytolysis, 98 and 99 per cent, described in one experiment above should be considered as a possible result of excess temperature. This may also have been associated with eggs that had a particular susceptibility to increased temperature or to the action of the solution of dye.

Results of the Study of Sections of Eggs from Pyronin Cultures

The chromosomes in the nonirradiated cultures were not modified; in the irradiated cultures after 15 minutes irradiation they showed pseudoamitosis and nuclear fragmentation (fig. 33). With longer irradiation (20 minutes) and greatly increased temperature (34.1° C.) the nucleus remained intact. With an irradiation of 20 minutes at a temperature of 29.4° C. there was failure of the pronuclei to unite. Figure 34 is from an egg fixed 56 minutes after insemination. The nuclei have not yet united.

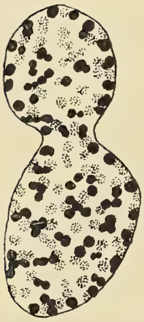


Fig. 33. Pyronin, 7/3/37, 1:55 P.M.

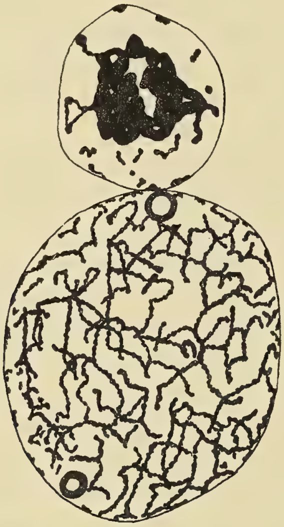


Fig. 34. Pyronin, 7/3/37, 12:48 P.M.

Rhodamine B

The rhodamine B used in this work was purchased from the Coleman and Bell Company. Rhodamine B is one of the xanthene dyes similar to pyronin, whose effect has just been considered. It is a basic dye; its solution is red in transmitted light and yellow in reflected light. Throughout this work it was used in concentrations of 1:25,000 and 1:50,000. In the stronger solution the dye was slightly toxic in diffuse-light cultures and was more toxic after irradiation. In the weaker solution it was nontoxic both in diffuse light and in irradiated cultures. In the five diffuse-light cultures in 1:50,000 rhodamine B that were studied, the eggs were diffusely stained and contained no deeply stained granules. The insemination-cleavage interval in one culture was of the same length as in the sea-water control, and in the other four cultures the eggs in the diffuse-light cultures in rhodamine B were from 1 to 2 minutes slower than in the sea-water control in beginning the first cleavage. Cleavage and development were regular. Development was followed as far as the swimming-blastula stage in only one culture. The temperature in these cultures varied from 27.6° to 28.6° C.

In the seven diffuse-light cultures in 1:25,000 solution of rhodamine B in sea water, there was some variation in the intensity of staining. All the cultures were alike in that the eggs in them were diffusely stained and showed no deeply stained granules. The insemination-cleavage interval in these eggs was, except in one instance, from 2 to 7 minutes longer than in the sea-water control. In the one exception, the interval was of the same length as in the sea-water control. In all the cultures in which this point was studied (and this includes the culture just mentioned in which the insemination-cleavage interval was the same in both dye culture and sea-water control), cleavage in the cultures in solutions of the dye lagged distinctly behind that in the control. Rhodamine B in this concentration, 1:25,000, may be said to be nontoxic during early cleavage, but it is not without effect later. During the late cleavages the toxic effects of the dye are evident. This is shown by the appearance of abnormal cleavages after the first division; for example, one blastomere may continue dividing while the other undergoes no further cleavage. The dye in this concentration seems to show toxicity in that many of the cleaving eggs disintegrate, and those that continue, develop only to swimming blastulae. These were less active and fewer than in the 1:50,000 cultures and remained in the blastula stage, while the eggs of the sea-water control developed to normal young plutei. The temperature in these cultures varied from 27.6° to 28.7° C., temperatures at which normal development may be expected.

In the 1:50,000 solution with 20-minute irradiation in sunlight, the eggs being stirred during irradiation, the temperature in the five cultures varied from 31.6° to 33.8° C. Irradiation in a solution of this strength might be described as ineffective when done at temperatures below 32° C. At the highest temperature, 33.8° C., there was little cleavage and some cytolysis. The same result was obtained at 32.6° C. At lower temperatures cleavage was regular, but the activity of the swimming blastulae seemed somewhat reduced.

The effect of irradiation in 1:25,000 solutions of rhodamine B in which the eggs were stirred for 20 minutes was more striking. In one of the six cultures of this class, cleavage was normal but slower than in the control. All the eggs in this culture had disintegrated on the following day. The temperature at which this culture was held during irradiation was 28.3° C. In the other cultures of this group, one, allowed to reach a temperature of 33.8° C. during irradiation, showed no divisions, although the nucleus had elongated and astral radiations were visible. Eggs in the culture allowed to reach 32.6° C. during irradiation divided to 2's but developed no farther. In eggs of the culture allowed to reach a temperature of 31.6° C. during irradiation, the first division was complete but only about 20 per cent reached the 4-cell stage. The results therefore vary from normal cleavage, slower than that in the control, with disintegration later, to no division, with disintegration within the first 2½ hours after insemination. A considerable degree of variation in the effect of irradiation in the 1:25,000 solution is evident. The irradiation may be described as effective because it checked development. No larvae developed so far as those in the 1:50,000 solution; none developed to the blastula stage. With irradiation of 10 minutes during which the eggs were stirred, the temperature reaching 31.3° C., the first division was normal but later division abnormal. In the same experiment, with irradiation of 15 minutes during which the eggs were stirred at a temperature of 28.1° C., cleavage was normal but disintegration occurred before the following day.

Results of the Study of Sections of Eggs from Rhodamine B Cultures

Sections were studied from eggs from experiments done with 1:25,000 solution of rhodamine B in sea water. In nonirradiated eggs the mitotic figure was normal; the chromosomes divided regularly.

In eggs irradiated for 10 minutes there was much variation in alignment of chromosomes. In general, however, this was irregular. The culture was more irregular in its development than the nonirradiated culture just described.

In one culture irradiated for 20 minutes the chromosomes were attenuated, and there were no globules. In the other cultures irradiated for 20 minutes the spindle was normal and contained compact, deeply stained chromosomes. There were deeply stained globules at the edges of the spindle and slight lagging in the distribution of the chromosomes.

FLUORESCEIN

Fluorescein (sodium salt), Coleman and Bell, was used throughout the experiments. In these experiments again, various concentrations of fluorescein were used on inseminated eggs without producing any effect. It seems probable that the dye did not pass through the fertilization membrane. No effect was found until uninseminated eggs were treated in the dye solutions.

The first seven sets of experiments were with inseminated eggs. The results are presented in the order of increasing concentration of the dye, although this is not the order in which the experiments were performed. The most dilute solution used was 1:150,000 in sea water. Inseminated *Lytechinus* eggs were transferred to 1:150,000 solution of fluorescein 6 minutes after insemination and kept in diffuse light.[1] Similarly, inseminated eggs were transferred to 1:150,000 solution of fluorescein 6 minutes after insemination and irradiated and stirred in sunlight for 10 minutes. The eggs in these two cultures cleaved perfectly, showing the same tempo of cleavage, differing from the eggs of the sea-water control only in the fact that the blastomeres were rather widely separated. These eggs gave rise to excellent plutei. Those of the irradiated culture were uniformly very large and active; those of the diffuse-light culture were not so large and showed greater variation. The plutei of both these cultures surpassed in size those of the sea-water control. On the second day after insemination these relative differences persisted. The plutei of the irradiated culture were uniform in size and activity and their second or anterior lateral arms were beginning to develop. The plutei of the diffuse-light culture were less uniform in size, but were equally active. The plutei of the sea-water control at this time, although normal and active, showed a less well advanced development of the anterior lateral arms than did those of the irradiated culture.

The next concentration used was a 1:100,000 solution in sea water. Inseminated *Lytechinus* eggs were transferred 5 minutes after insemination to a solution of fluorescein of this concentration at a temperature of 28.4° C. and kept in diffuse light. Under a high dry objective no evidence of entrance of the stain could be found. These eggs cleaved regularly in the tempo of those of the sea-water control, the first division beginning 37 minutes after insemination. They gave rise to normal blastulae and gastrulae and excellent plutei. Inseminated eggs from the same stock culture were transferred 5 minutes after insemination to 1:100,000 solution of fluorescein, and irradiated and stirred for 20 minutes, the temperature at the end of this time being 28.6° C. The eggs remained unstained and developed regularly in the same manner as those of the diffuse-light control. They gave rise to normal blastulae and gastrulae and excellent plutei.

Inseminated eggs transferred to a 1:75,000 solution of fluorescein in sea water 10 minutes after insemination and kept in diffuse light divided regularly like those in the sea-water control and developed to fine, active, large plutei. Inseminated eggs of the same lot, transferred to 1:75,000 fluorescein 10 minutes after insemination, and irradiated and stirred for 10 minutes, developed like the eggs of the diffuse-light culture. The blastulae of both the diffuse-light and the irradiated cultures were all swimming at the surface 6½ hours after insemination. Those of the sea-water control were not beginning to leave the bottom of the culture dish until an hour later. They were still streaming to the surface 8½ hours after insemination. On the following day the plutei of both the diffuse-light and the irradiated cultures were fine and active, and larger than those of the control, which were normal and active.

Inseminated *Lytechinus* eggs were transferred to 1:50,000 solution of fluorescein 5 minutes after insemination and kept in diffuse light at 28.4° C. The eggs remained unstained. They began to divide 38 minutes after insemination, and their cleavage and later development were as regular as in the sea-water control.

[1] Temperature data for fluorescein experiments in 1938 could not be found, but the temperature was presumably 30° ± 3° C.—W. L. D.

They gave rise to normal, fine plutei. Inseminated eggs of the same lot, transferred 5 minutes after insemination to 1:50,000 solution of fluorescein in sea water, and irradiated and stirred for 20 minutes, with the temperature held at 28.6° C., remained unstained like those previously described. They cleaved regularly and developed normally to plutei which were somewhat more stumpy than those of the sea-water control and the diffuse-light culture.

Two series of experiments were performed with 1:37,500 solutions of fluorescein in sea water. In the first of these, inseminated *Lytechinus* eggs were transferred to the solution of fluorescein 10 minutes after insemination and kept in diffuse light. These eggs showed a slightly greenish color but there was no evidence of entrance of stain into the egg. Cleavage in this culture was somewhat slower than in the sea-water control or in the irradiated culture. The eggs developed to excellent late gastrulae, but died before reaching the pluteus stage. The only reason that can be given for the more rapid development of the irradiated culture is that during irradiation the temperature of the culture was raised slightly. Inseminated eggs transferred to 1:37,500 solution of fluorescein in sea water 10 minutes after insemination, and irradiated and stirred for 10 minutes, divided regularly into 2 cells and appeared slightly greenish in color. In their later cleavage these eggs were slightly more rapid than those of the diffuse-light culture. They developed to excellent gastrulae and fine plutei.

Because of the somewhat unsatisfactory results in these cultures, the experiments were repeated. Inseminated *Lytechinus* eggs were transferred to 1:37,500 solution of fluorescein in sea water 10 minutes after insemination and kept in diffuse light. These eggs showed no evidence of stain and developed slightly more rapidly than those of the sea-water control. Inseminated eggs from the same culture were transferred to 1:37,500 solution of fluorescein in sea water 10 minutes after insemination, and irradiated and stirred for 10 minutes. These eggs also developed somewhat more rapidly than those of the sea-water control. On the following morning the plutei of the diffuse-light, the irradiated, and the sea-water control cultures were similar and normal. The mouth in none of these plutei had opened. The cultures were all good. There was no evidence that the eggs were permeable to fluorescein in this concentration.

A series of experiments using fluorescein in 1:18,750 concentrations was then performed. Inseminated *Lytechinus* eggs were transferred to a solution of fluorescein of this strength 10 minutes after insemination. The eggs cleaved regularly, during their earlier development surpassing, in uniformity and advancement, those of the control. Inseminated eggs transferred to 1:18,750 solution of fluorescein in sea water 10 minutes after insemination, and irradiated and stirred for 10 minutes, cleaved like those of the diffuse-light culture and, like these, surpassed the eggs of the sea-water control in uniformity and advancement. On the following morning, however, 26 hours after insemination, the diffuse-light culture and the irradiated culture contained only late gastrulae and prism larvae, whereas the sea-water control contained young plutei.

Before proceeding with the discussion of the effects of fluorescein on uninseminated eggs, it is desirable to mention an experiment in which the effects of pre-irradiated 1:10,000 solution of fluorescein were compared with those of nonirradiated solution of the same strength. Inseminated *Lytechinus* eggs were transferred to 1:10,000 nonirradiated solution of fluorescein 5 minutes after insemination and kept in diffuse light. The eggs remained spherical and appeared slightly yellow in transmitted light. The eggs looked slightly more green in reflected light than those of the succeeding culture. Inseminated *Lytechinus* eggs of the same lot were transferred to 1:10,000 pre-irradiated solution of fluorescein 5 minutes after insemination. The solution used had been pre-irradiated for 2 hours, during which much of the green, the color in reflected light, was lost. The eggs in this solution also remained spherical and looked slightly yellow in transmitted light. Both the diffuse-light culture and the culture in pre-irradiated solution of fluorescein gave perfect cleavage stages, no difference between them being evident. Both were followed to the swimming-blastula stage.

All the experiments with uninseminated eggs were done with 1:50,000 solution of fluorescein in sea water. In the first of these, uninseminated *Lytechinus* eggs were transferred to 1:50,000 solution of fluorescein in sea water in diffuse light at a temperature of 28.6° C. The eggs were inseminated in this solution 23 minutes later. The surface of the eggs at the time of fertilization was smooth, and the fertilization membrane was uniformly separated. The eggs in this culture began to divide 42 minutes after insemination. Cleavage proceeded regularly, resulting in normal blastulae and gastrulae. On the following morning, 21 hours after insemination, these cultures showed good young plutei.

Uninseminated eggs from the same culture were transferred to 1:50,000 solution of fluorescein in sea water, and irradiated and stirred for 20 minutes with the temperature held at 28.7° C. These eggs were inseminated, in the solution in which they were irradiated, 3 minutes after the close of irradiation. Before insemination the surface of the eggs was slightly rough. No fertilization membranes were separated, but fertilization stages were followed under high magnification (4D), the union of the nuclei being seen 9 minutes after insemination. Twenty minutes after insemination the surface of these eggs was irregularly indented. The plasma membrane was irregular. The fertilization membrane was slightly separated in patches in some eggs. During the next 10 minutes indentation of the eggs increased. Ten minutes later, or about 48 minutes after insemination, all the eggs were indented, but many were cleaving. A close fertilization membrane could be seen extending over the groove between the blastomeres. Most of the eggs cleaved to the 2-cell stage, all being pitted; some remained undivided but were also pitted. During their later cleavage the eggs in this culture remained compact and solid. Little in the nature of a cleavage cavity could be seen. On the following morning, 21 hours after the beginning of the experiment, all the cultures showed only aberrant blastulae and gastrulae, whereas, as has been noted, the eggs in the diffuse-light culture had formed young plutei.

These experiments were repeated with eggs from another female. Uninseminated *Lytechinus* eggs were transferred to 1:50,000 solution of fluorescein in sea water, inseminated in this solution 20 minutes later at a temperature of 29.2° C., and kept in diffuse light. The eggs formed excellent fertilization membranes;

they divided to 2 cells 44 minutes after insemination. On the following morning, 17 hours after insemination, development had proceeded to the gastrula stage; in the control there were many good young plutei.

Uninseminated eggs transferred to 1:50,000 fluorescein, and irradiated and stirred for 20 minutes with the temperature held at 29.4° C., were inseminated 3 minutes after the end of irradiation. These eggs formed no separated fertilization membranes. Many of them became pitted 24 minutes after insemination. They began to constrict for the first division 40 minutes after insemination, and 5 minutes later most eggs were in very compact 2-cell stages, the fertilization membrane showing over the groove between the blastomeres. On the following morning, 17 hours after insemination, all the embryos from these eggs, which meantime had developed to stereoblastulae, were dead.

Uninseminated *Lytechinus* eggs, transferred to 1:50,000 solution of fluorescein and irradiated and stirred for 20 minutes, were inseminated 3 minutes after the end of irradiation. The culture was not cooled during irradiation and its temperature rose to 34.2° C. It should be noted that this temperature is excessive. These eggs formed no separated fertilization membranes, but the union of the nuclei was followed with the microscope. The eggs at first showed less pitting than in the culture just described. About 38 minutes after insemination, pitting was common. An hour after insemination, the culture showed eggs in compact 2-cell and partial 2-cell stages as well as undivided eggs. On the following morning all the eggs in this culture were dead, development never having proceeded beyond early cleavage stages.

These experiments show conclusively that the fertilization membrane prevents the entrance of fluorescein into the egg. When the unfertilized egg is transferred to solution of fluorescein in sea water and irradiated before insemination, the result is very similar to that which follows the irradiation of unfertilized eggs in solutions of eosin (see below). The results were the same in all the experiments. If the cultures were not irradiated, normal development took place in the cultures in fluorescein, but if the cultures were irradiated with sunlight, the temperature being held close to that of the sea water, aberrant development followed.

In 1939 some work with fluorescein was repeated in order to get data on intensity of sunlight. The following table gives the results.

Summary of Results of Irradiation of Eggs in Solutions of Fluorescein for Various Periods of Time

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/19/39............	(1)	28.8	1:80,000	0	Dark
	(2)	30.6	1:80,000	15	7969
	(3)	28.8	1:40,000	0	Dark
	(4)	30.9	1:40,000	15	7969

Results. (1) 6 minutes after insemination well separated membranes, no change of surface of eggs; 39 minutes after insemination, into 2's; 1 hour and 39 minutes after insemination 4's, 8's, 12's, 16's; 3 hours and 19 minutes after insemination excellent hollow spheres, 128 cells plus; 5 hours and 43 minutes after insemination blastulae beginning to swim; on the following morning many good plutei, other stages from gastrulae up.

(2) 7 minutes after insemination poorly separated membranes; slight roughening of eggs during next 26 minutes, surface slightly but frequently pitted; 39 minutes after insemination 50 per cent into 2's, a good deal of shallow pitting; 99 minutes after insemination irregular and tight 4's, 8's, 12's, 16's; 5 hours and 41 minutes after insemination highly irregular late cleavage; on the following morning all were disintegrated.

(3) 7 minutes after insemination well separated membranes; during next 20 minutes surface remained unmodified; 40 minutes after insemination into 2's; 1 hour and 41 minutes after insemination excellent 4's, 8's, 12's, 16's; 3 hours and 19 minutes after insemination excellent hollow spheres 128 cells plus; 5¾ hours after insemination blastulae beginning to swim; on the following morning plutei, gastrulae, and younger, much disintegration.

(4) 8 minutes after insemination 1 poorly separated membrane out of over 200 eggs; only change during next 18 minutes is slight roughening of surface of eggs; surface slightly but frequently pitted; 41 minutes after insemination, into 2's, a good deal of shallow pitting; 1 hour and 42 minutes after insemination 2's, 4's, 8's, 12's, 16's (50 per cent), remainder 1's; 3 hours and 19 minutes after insemination 50 per cent in cleavage, 50 per cent rather poor cleavage, 128 cells plus; 5 hours and 46 minutes after insemination 50 per cent highly irregular late cleavage, others no development; on the following morning stereoblastulae and disintegrated eggs, some intact eggs.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/19/39............	(1)	30.6	1:80,000	0	Dark
	(2)	31.4	1:80,000	20	8500

Results. (1) 14 minutes after insemination narrowly separated fertilization membranes; 29 minutes after insemination 75 have separated membranes; all those with separated membranes are irregular in outline; 1 hour after insemination 50 per cent are in 2's; 1 hour and 17 minutes after insemination 50 per cent are in cleavage, now in 4's; on the following morning gastrulae and prism larvae.

(2) 14 minutes after insemination no separated membranes; 30 minutes after insemination no separated membranes, 50 per cent are irregular in outline; 59 minutes after insemination 33 per cent in 2's; 1 hour and 17 minutes after insemination 30 per cent in cleavage, now in 4's; on following morning gastrulae, disintegrated eggs, and unfertilized eggs.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/20/39............	(1)	29.6	1:80,000	0	Dark
	(2)	30.2	1:80,000	25	8633–8766–9031–9297
	(3)	29.6	1:40,000	0	Dark
	(4)	30.1	1:40,000	25	8633–8766–9031–9297

Results. (1) 2's and into 2's 39 minutes after insemination; unsatisfactory because of poor eggs.

(2) 39 minutes after insemination 10 per cent 2's and into 2's; a few pits and vacuoles were formed; there was also about 25 per cent lobular cytolysis; unsatisfactory because of poor eggs.

(3) 40 minutes after insemination 2's and into 2's; unsatisfactory because of poor eggs.

(4) 40 minutes after insemination about 1 per cent cleavage; a few pits and vacuoles were formed; about 25 per cent lobular cytolysis; unsatisfactory because of poor eggs.

Results of the Study of Sections of Eggs from Fluorescein Cultures

The eggs that were fixed in 1935 were inseminated before irradiation for 10 minutes in 1:37,500 solution of fluorescein. There was no evidence that these eggs were permeable to the dye. In both the nonirradiated and the irradiated cultures division was normal, the chromosomes being very sharply stained. The number of chromosomes in both cultures was less than normal, but this was also true of the eggs of the control in sea water.

The eggs that were fixed in 1937 were inseminated after irradiation for 20 minutes in 1:50,000 solution of fluorescein. In these eggs the chromosomes were rather diffusely stained. It was difficult to distinguish between chromosomes and spindle fibers. There were usually one or more deeply stained spherules on the spindle. The number of chromosomes was lessened. There was considerable lagging in division.

It was thus evident that the dye had a greater effect when used on uninseminated eggs.

EOSIN Y

The Coleman and Bell Company's eosin Y, certified, dye content 89 per cent, was used in this work. Experiments were performed with both inseminated and uninseminated eggs.

In the first experiments inseminated eggs were transferred 10 minutes after insemination to 1:100,000 solution of eosin Y in sea water and kept in diffuse light. A similar culture was irradiated and stirred for 10 minutes. At no time was any effect of eosin Y evident in either of these cultures. There was no evidence that the dye had entered the eggs. The eggs in the sea-water control and in both these cultures cleaved similarly and were followed through the gastrula to the pluteus stage. The eggs in the experimental cultures appeared to be exactly the same as those in the control cultures.

Three cultures in 1:50,000, 1:25,000, and 1:12,500 solutions of eosin Y in sea water were then made. *Lytechinus* eggs were transferred 5 minutes after insemination to 1:50,000 solution of eosin Y and kept in diffuse light. Similarly, eggs were transferred to 1:50,000 solution of eosin Y 5 minutes after insemination, and irradiated and stirred for 20 minutes. Both these cultures behaved like the control. The eggs in all were beginning to enter the 2-cell stage 37 minutes after insemination and the 4-cell stage 21 minutes later. With the 1:25,000 cultures, inseminated eggs that were transferred to the solution of eosin and kept in diffuse light were entering the 2-cell stage 40 minutes after insemination. Those that were transferred to this solution and irradiated and stirred for 20 minutes became slightly irregular in form, but entered the beginning 2-cell stage 40 minutes after insemination, just as had those in the solution kept in diffuse light.

Inseminated *Lytechinus* eggs that were transferred to 1:12,500 solution of eosin 5 minutes after insemination and kept in diffuse light entered the 2-cell stage 40 minutes after insemination. Eggs transferred to solution of eosin Y, 1:12,500, and irradiated and stirred for 20 minutes showed only a scattered few entering the 2-cell stage 40 minutes after insemination, while many were blistering. About half of these eggs cytolized during the first 2 hours after insemination. Many did not begin cleavage; none went beyond the 2-cell stage.

In another series, inseminated eggs, mixed from several females, were transferred to 1:25,000 solution of eosin Y in a diffuse-light culture at 28.5° C. These eggs remained spherical and formed normal amphiasters, and some began to cleave 36 minutes after insemination. The eggs of the control showed the same insemination-cleavage interval. The eggs in the diffuse-light culture cleaved regularly and gave rise to plutei which were less good than those of the control. Inseminated eggs that were transferred to 1:25,000 eosin Y and irradiated and stirred for 12 minutes, the temperature at the end of this time being 32.3° C., gave rise to normal cleavage stages, the rhythm of cleavage being the same as in the control and in the diffuse-light culture just described. These eggs gave rise to plutei which were less good than the ones described above in that most failed to develop normal arms.

A series of experiments was then tried with 1:12,500 concentrations. Inseminated eggs transferred to a solution of this concentration in diffuse light at 29.5° C. kept a fine spherical form. They showed excellent mitotic figures and divided to good 2-cell stages 44 minutes after insemination. The eggs of the sea-water control were similarly slow in their first division. Inseminated eggs that were irradiated and stirred for 20 minutes in 1:12,500 solution of eosin Y in sea water, during which the temperature reached 33.9° C., retained their spherical form and showed good mitotic figures. Many of these eggs divided, some being poorly constricted 45 minutes after insemination. Many did not divide, thus being unlike the eggs of the control and of the diffuse-light cultures. Some of the undivided eggs blister cytolized. There was some disintegration as well as blister cytolysis during the next 10 minutes. All, experimental cultures as well as control, showed regular 4- and 8-cell stages. On the morning of the following day the sea-water control contained excellent prism larvae, whereas the irradiated culture showed very abnormal gastrulae with abnormal mesenchyme and many exogastrulae. The nonirradiated dye culture was less abnormal than the irradiated.

A series of cultures was then tried with more dilute solutions of eosin Y in sea water, 1:150,000. Inseminated *Lytechinus* eggs were transferred to 1:150,000 eosin Y in neutral sea water 5 minutes after insemination. In the diffuse-light cultures no effect on the eggs was evident; no stain was visible in them. The surface of the eggs was slightly more irregular than in the control. They were somewhat indented, but became smooth within half an hour. It is possible that this roughening and indentation of the surface was an effect of the neutral sea water used in the experiment. The eggs in this culture cleaved regularly, but somewhat more slowly than those of the sea-water control. Inseminated *Lytechinus* eggs were transferred to 1:150,000 solution of eosin Y in neutral sea water 5 minutes after insemination and were irradiated and stirred for 20 minutes with the temperature held at 28° C. These eggs likewise became irregular and indented. Like the eggs described above, they became smooth and proceeded through a regular cleavage that resembled that of the culture kept in diffuse light. The sky became overcast immediately after irradiation of this and the succeeding culture began. For this reason the third experimental culture was irradiated and stirred for 30 minutes, the temperature being held at 28° C. The eggs of this culture behaved in all respects like those described above. These experiments cannot be regarded as satisfactory because of the insufficient intensity of irradiation.

Inseminated *Lytechinus* eggs transferred 5 minutes after insemination to solutions kept in diffuse light at a temperature of 28.9° C. were irregular and indented 7 minutes after transfer. These eggs were slightly slower in beginning cleavage than those of the sea-water control, and gave rise to irregular blastomeres. Inseminated eggs transferred to eosin Y, 1:150,000, in neutral sea water 5 minutes after insemination, and irradiated and stirred for 15 minutes with the temperature controlled at 28.5° C., behaved exactly like those just described. Similar eggs irradiated and stirred for 20 minutes with the temperature held at 29.2° C. became irregular and indented, and gave rise to cleavage stages with irregular blastomeres as described above. Finally, inseminated eggs transferred to eosin Y solution 5 minutes after insemination, and irradiated and stirred for 20 minutes without cooling, with the temperature rising to 32.8° C., behaved in all respects like the cultures just described.

All the experiments described above were performed with inseminated eggs. The most pronounced effects were found following the use of 1:12,500 solutions. Even in solutions of this strength there was little evidence of the passage of dye through the fertilization membrane. Experiments were therefore undertaken with uninseminated eggs. In the first of these, *Lytechinus* eggs were transferred to 1:150,000 solution of eosin Y and kept in diffuse light at 28.3° C. The eggs were inseminated in this solution 20 minutes after transfer. They formed good fertilization membranes, divided in the same time as those of the sea-water control, showed no vacuoles in the cytoplasm, and gave rise to late cleavage stages similar to those of the sea-water control. Uninseminated eggs transferred to 1:150,000 solution of eosin Y in sea water, and irradiated and stirred for 20 minutes with the temperature held at 28.1° C., were inseminated in the dye solution at the end of irradiation. These eggs formed no separated fertilization membranes. They were slow in beginning constriction for division, and many showed the presence of small vacuoles, containing rounded concretions, in the cytosome. The eggs containing vacuoles began to cytolize an hour after insemination. None of the eggs in this culture divided normally; more than 10 per cent became vacuolated and disintegrated. Many of the eggs formed irregular protuberances.

Similarly, uninseminated *Lytechinus* eggs were transferred to 1:75,000 solution of eosin Y in sea water and kept in diffuse light at 28.3° C. These eggs were inseminated 20 minutes after transfer to the solution. They formed good fertilization membranes and were constricting for the first division 37 minutes after insemination. They formed good 2-cell stages and continued cleavage as did those in the sea-water control. No formation of vacuoles and no irregularity of cleavage was found. Uninseminated *Lytechinus* eggs were transferred to 1:75,000 solution of eosin Y in sea water, irradiated and stirred for 20 minutes at a temperature of 28.1° C., and inseminated at the end of irradiation. These eggs formed no separated fertilization membranes. Many eggs showed pink vacuoles containing refringent bodies. These eggs with vacuoles began to cytolize, and their number increased greatly until more than half were vacuolated. Each vacuole contained from 1 to 15 refringent bodies. None of these eggs cleaved.

It seems evident from the above that the stronger solution, 1:75,000, is somewhat more effective in the production of vacuoles than is the weaker solution, 1:150,000. The point of interest in both these experiments is that formation of vacuoles is common in the eggs transferred to solutions of dye before insemination. In the previously described experiments the fertilization membrane apparently prevented the entrance of the solution of dye into the egg.

In the next series of experiments the sky became overcast just as irradiation was begun, and the duration of irradiation was therefore prolonged to 30 minutes in two of the experiments. Uninseminated eggs were transferred to 1:150,000 solution of eosin Y and after 20 minutes in diffuse light at a temperature of 28.5° C. were inseminated. These eggs formed excellent fertilization membranes, cleaved regularly, and formed swimming blastulae in the tempo of the eggs in the sea-water control. Uninseminated eggs transferred to 1:150,000 solution of eosin Y, irradiated and stirred for 20 minutes at a temperature of 28.3° C., and inseminated at the end of irradiation formed no separated fertilization membranes, although the union of the germ nuclei was observed 7 minutes after insemination. These eggs showed much slight pitting of the surface, and 30 minutes after insemination about 2 per cent were blistered. Some cleaved to 2 cells, the blastomeres remaining very closely applied to each other. Many did not divide. All had become vacuolated about 3 hours after insemination, and within 6 hours after insemination only vacuolated and cytolizing eggs were to be found in the culture.

Uninseminated eggs of the same lot transferred to 1:150,000 solution of eosin Y, irradiated and stirred for 30 minutes with the temperature held at 28.4° C., and inseminated at the end of irradiation showed a distinct fertilization membrane in close contact with the egg. Many of these eggs blistered; some contained vacuoles; a few began cleavage. Ultimately all the eggs of this culture became vacuolated and cytolized. Uninseminated eggs transferred to 1:150,000 eosin Y, and irradiated and stirred for 30 minutes, the temperature not being controlled and rising to 31.9° C., were inseminated at the end of irradiation. These eggs were fertilized, but fertilization membranes did not separate; a few eggs were badly blistered within 7 minutes after insemination; within the following 10 minutes blistering increased greatly, until fully 10 per cent of the eggs were blistered. Blistering was somewhat asymmetric, a clear protrusion arising from one region of the egg. Within 20 minutes after insemination all the blistered eggs became spherical masses of globules; the remainder of the eggs showed tight and somewhat irregular cleavage. Finally all the eggs became badly vacuolated and cytolized.

In 1939 some work on eosin Y was repeated for photographic purposes and in order to get data on the effect of intensity of sunlight. The results are given in the following table.

Summary of Effects of Light Intensity, Dye Concentration, and Period of Irradiation on Eggs in Solutions of Eosin Y

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/18/39............	(4)	29.0	1:50,000	0	Dark
	(5)	31.6	1:50,000	10	6906–7305
	(6)	31.8	1:50,000	15	6906–7305

Results. (4) 9 minutes after insemination well separated fertilization membranes; 21 minutes after insemination all with well separated fertilization membranes; 40 minutes after insemination 75 per cent in 2's, others into 2's; 53 minutes after insemination 2-cell, plate 4, figure 6; 54 minutes after insemination 2-cell with blebs; 2 hours after insemination 100 per cent regular 16's, 24's, 32's; on the following morning prism larvae.

Control, plate 5, figure 1, 60 minutes after insemination.

(5) 10 minutes after insemination no separated fertilization membranes; 17 minutes after insemination tight fertilization membranes on most; 38 minutes after insemination many in constriction; 49 minutes after insemination 85 per cent in 2's, rough surface; 55 minutes after insemination 2-cell, blebs; plate 5, figure 2 shows 3 eggs at 4D 65 minutes after insemination; 70 minutes after insemination 4-cell, blebs disappearing; 2 hours after insemination very irregular 4's and 8's, no blebs; on the following morning some rotating blastulae.

(6) 6 minutes after insemination no fertilization membranes, 1 per cent pitting and vacuoles; 13 minutes after insemination radiations around nucleus but no separated fertilization membranes; 34 minutes after insemination 50 per cent with rough surface, rare constriction; 43 minutes after insemination 2 per cent in 2's; 53 minutes after insemination 2-cell, blebs; 68 minutes after insemination 1's, 2's, 4's, blebs disappearing; 1 hour and 54 minutes after insemination 1's, irregular 1's, 2's, 3's, 4's, 7's, 8's, etc., no blebs; on the following morning some rotating blastulae.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/19/39............	(3)	30.6	1:80,000	0	Dark
	(4)	31.8	1:80,000	20	8500

Results. (3) 14 minutes after insemination a few narrowly separated fertilization membranes; 32 minutes after insemination a few narrowly separated membranes, these eggs irregular in form; 57 minutes after insemination 10 per cent in 2's; on the following morning gastrulae, stereoblastulae, disintegrated unfertilized eggs.

(4) 14 minutes after insemination no membranes; 34 minutes after insemination many pitted and vacuolated, no separated membranes; 57 minutes after insemination no 2's; on the following morning disintegrated eggs, also unfertilized eggs stretched out of shape and adhering to one another.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/20/39............	(1)	29.4	1:100,000	0	Dark
	(2)	31.2	1:100,000	20	7969–8633
	(3)	29.4	1:50,000	0	Dark
	(4)	31.1	1:50,000	20	7969–8633

Results. (1) 3 minutes after insemination very few with separated membranes; 78 minutes after insemination 2's, 4's, no pits or vacuoles.

(2) 4 minutes after insemination no separated membranes, 10 per cent pits and vacuoles; plate 5, figure 3, 18 minutes after insemination, shows pits and vacuoles; 79 minutes after insemination 15 per cent lobular cytolysis.

(3) 7 minutes after insemination a few separated membranes; 80 minutes after insemination 2's, 4's, and unfertilized eggs.

(4) 9 minutes after insemination pits and vacuoles; plate 5, figures 4 and 5, 20 and 22 minutes after insemination, show pits and vacuoles; 80 minutes after insemination 35 per cent lobular cytolysis, pits and vacuoles gone.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/21/39............	(3)	29.2	1:100,000	0	Dark
	(4)	30.6	1:100,000	20	8633

Results. (3) 5 minutes after insemination 100 per cent separated membranes; 50 minutes after insemination in 2's.

(4) 5 minutes after insemination no membranes; 48 minutes after insemination disintegration; 2's and unfertilized eggs.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/22/39............	(3)	28.2	1:150,000	0	Dark
	(4)	30.8	1:150,000	20	8633

Results. (3) 6 minutes after insemination 95 per cent separated membranes; 13 minutes after insemination 95 per cent separated membranes.

(4) 7 minutes after insemination no separated membranes; 16 minutes after insemination blebs; 38 to 48 minutes after insemination photographs taken at minute intervals showing blebs, plate 5, figure 6 taken 47 minutes after insemination; cups in surface filled with

eosin-colored solution, then vacuoles; expansion from surface, then blebs; great increase in number of blebs, one large then many small; then mass of small blebs that surround core with a cortex of uniform-sized vesicles; 66 minutes after insemination 12 per cent in 2's, 20 per cent cytolized.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/23/39............	(3)	29.0	1:150,000	0	Dark
	(4)	30.5	1:150,000	20	7969

Results. (3) 6 minutes after insemination strong, well separated membranes; 44 minutes after insemination, into 2's; 1 hour and 55 minutes after insemination 4's, 8's, 12's, and 16's.

(4) 4 minutes after insemination no separated membranes; 1 hour and 55 minutes after insemination 1 per cent irregular cleavage, 10 per cent lobular cytolysis.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/24/39............	(1)	28.6	1:150,000	0	Dark
	(2)	30.0	1:150,000	20	8633–8766–9031–9297

Results. (1) 4 minutes after insemination well separated membranes; plate 6, figure 2, 22 minutes after insemination.

(2) 4 minutes after insemination no separated membranes; 12 minutes after insemination eggs with vacuoles; 13 to 21 minutes after insemination cups; 17 to 61 minutes after insemination, plate 6, figures 1 and 3 to 6.

Results of the Study of Sections of Eggs from Eosin Y Cultures

The material from which sections were prepared was from three sources: first, inseminated eggs transferred to 1:25,000 eosin Y; second, inseminated eggs transferred to 1:12,500 eosin Y; and third, uninseminated eggs transferred to 1:150,000 eosin Y, irradiated, and inseminated at the end of irradiation.

In the fertilized eggs placed in 1:12,500 solution of eosin Y and allowed to remain in diffuse light, segmentation was normal and regular. There was no lagging of chromosomes, although there was occasionally some irregularity of alignment. No nucleoli were evident. Sections of inseminated eggs irradiated for 20 minutes in 1:12,500 solution of eosin Y, during which the temperature rose to 33.9° C., showed at most slight lagging of chromosomes. They showed during division small, deeply stained globules at the edges of the spindle.

Sections of inseminated eggs placed in 1:25,000 solution of eosin Y and not irradiated divided normally. Sections of eggs irradiated for 12 minutes in 1:25,000 solution of eosin Y, during which the temperature rose to 32.3° C., showed chromosomes sharply stained and very slight lagging in division. No globules or nucleoli were present on the spindle.

The only difference between these cultures lay in the presence of nucleoli on the spindle in eggs in the stronger solution.

In the eggs placed in 1:150,000 solution of eosin Y in sea water, irradiated, and inseminated at the close of irradiation, the results were different. Eggs placed in the eosin Y solution and inseminated 10 minutes later developed normally, showing at most very slight irregularity of alignment. Eggs irradiated for 20 minutes, cooled during the process so that the temperature never rose above 28.3° C., and inseminated at the close of irradiation showed many nucleolus-like bodies during prophase (globules which disappeared during anaphase). Although no effects on the division figure were evident, the fact that there had been an upset was apparent 3 hours later when the eggs contained from 32 to 64 nuclei. There was then a sheet of nuclei or of mitotic figures just below the surface of the eggs. There were many deeply stained spherules on the spindles, no normal chromosomes being visible. When the nonïrradiated eggs were swimming blastulae, the irradiated eggs were cytolizing.

Uninseminated eggs that were irradiated in 1:150,000 solution of eosin Y and cooled during the process, the temperature being held at 28.4° C., and inseminated at the end of irradiation showed pitting and blistering 40 minutes after insemination. Many of these eggs were in anaphase. Three hours later they contained from 32 to 64 nuclei as large as the segmentation nucleus in undivided eggs. There had been a large increase in nuclear material although there was no evidence of mitotic figures and no indication of cytoplasmic division. These nuclei were almost in contact. They lay in numerous groups of 2 or 3 nuclei formed as the result of diastral and triastral divisions.

Uninseminated eggs irradiated for 30 minutes in 1:150,000 solution, the temperature rising to 31.9° C. during this process, were inseminated at the end of irradiation. These eggs were slightly in advance of those just described, probably because of the increased temperature. Blistering began within 5 minutes after the end of irradiation and increased rapidly within the following 40 minutes, when all the blistered eggs had become spherical masses of globules while the remainder of the eggs showed tight and somewhat irregular cleavage. These eggs that had been subjected to a temperature of 31.9° C. showed several deeply stained globules along the edges of the spindles 40 minutes after insemination. Five and a half hours after insemination, when the eggs of the control and the nonirradiated culture were swimming blastulae, all of these irradiated and inseminated eggs that were still alive were badly vacuolated and cytolizing.

PHLOXINE

Phloxine, Strohmeyer and Lauth, was used in 1:40,000 solution. Within 7 minutes after the end of 15-minute irradiation 10 per cent of the eggs were pitted and vacuolated. In some instances the vacuoles united. This vacuolization increased during the next 12 minutes to 15 per cent, and to 50 per cent during the next half-hour, the vacuoles having a pink color. During this time most of the eggs formed blisters of the neutral red type. Finally all the irradiated eggs disintegrated. About 10 per cent of the nonirradiated eggs remained intact, the others disintegrated.

The results of this work are given in the following table.

Summary of Results of Irradiation of Eggs in Solutions of Phloxine 1:40,000

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/16/39............	(1)	28.8	1:40,000	0	Dark
	(2)	30.8	1:40,000	10	9297–9562–9828
	(3)	30.1	1:40,000	15	9297–9562–9828

Results. (1) 3 minutes after insemination no separated membranes; 15 minutes after insemination no vacuoles, pits, or separated membranes; 21 minutes after insemination 2 per cent pits and vacuoles, rare fertilization membranes; 30 minutes after insemination pitting and vacuolization not increased; control 34 minutes after insemination first constriction appeared; control 37 minutes after insemination about 50 per cent in 2's; (1) 40 minutes after insemination no pits, no vacuoles, no separated membranes; 1 hour and 20 minutes after insemination eggs appear normal; 3 hours after insemination eggs appear normal; 4¼ hours after insemination 1 per cent cytolizing, some with vacuoles; on the following morning 10 per cent of eggs intact, others disintegrated.

(2) 4 minutes after insemination no separated membranes; 12 minutes after insemination 1 to 2 per cent pitting and vacuoles; 22 minutes after insemination 2 per cent pits and vacuoles; 31 minutes after insemination pitting and vacuolization not increased; 38 minutes after insemination 1 per cent blister cytolysis of the neutral red type; 1 hour and 20 minutes after insemination 2 per cent pits and vacuoles; 3 hours after insemination 25 per cent pits and vacuoles; 4¼ hours after insemination 25 per cent pits and vacuoles, no cytolysis; on the following morning all disintegrated.

(3) 4 minutes after insemination 2 per cent pitting and vacuoles, no fertilization membranes; 5 minutes after insemination 5 per cent pitting and vacuoles; 6 minutes after insemination 10 per cent pitting and vacuoles, in some vacuoles are uniting; 18 minutes after insemination 15 per cent pits and vacuoles; 31 minutes after insemination about 1 per cent blister cytolysis of the neutral red type; 1 hour and 17 minutes after insemination 20 per cent pits and vacuoles; 3 hours after insemination 50 per cent pits and vacuoles, vacuoles pink; 4 hours after insemination 50 per cent pitting and vacuolization, most of eggs have small blisters, color gone from eggs that are cytolizing; on the following morning all were disintegrated.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/17/39............	(4)	28.8	1:40,000	0	Dark
	(5)	30.1	1:40,000	10	7172–7437–7703–7969
	(6)	30.1	1:40,000	15	7172–7437–7703–7969

Results. (4) 11 minutes after insemination no fertilization membranes, appearance normal; 18 minutes after insemination appearance normal, some with membranes; 25 minutes after insemination about 10 per cent with fertilization membranes; 42 minutes after insemination about 10 per cent show nuclear activity, 2 per cent in 2-cell stage; photographs of (4), (5), and (6) taken; 64 minutes after insemination, plate 7, figure 1; 3 hours and 27 minutes after insemination 10 per cent hollow spheres, others unchanged; 4 hours and 26 minutes after insemination hollow spheres and unstained eggs; on the following morning young plutei and undivided eggs.

(5) 12 minutes after insemination no fertilization membranes, some pitted, some stained and blistered; 18 minutes after insemination rare staining of eggs; 26 minutes after insemination 5 per cent vacuolated; 43 minutes after insemination 2 per cent vacuolated; 3 hours and 29 minutes after insemination 10 per cent vacuolated, some vacuoles have run together; 4 hours and 27 minutes after insemination 10 per cent vacuolated; on the following morning 75 per cent of eggs have retained their form and appearance, 25 per cent have cytolized with or without color.

(6) 8 minutes after insemination some, very few, pitted; 13 minutes after insemination rare swelling of eggs; 22 minutes after insemination rare swelling and staining; 38 minutes after insemination 10 per cent vacuolated, vacuoles pink; 64 and 65 minutes after insemination, plate 7, figures 2 and 3, respectively; 3 hours and 25 minutes after insemination 10 per cent vacuolated; 4 hours and 24 minutes after insemination 10 per cent vacuolated; on the following morning 95 per cent have cytolized with or without color, 5 per cent have retained normal appearance.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/18/39............	(1)	29.0	1:40,000	0	Dark
	(2)	30.0	1:40,000	10	6906–7305
	(3)	30.2	1:40,000	15	6906–7305

Results. (1) 4 minutes after insemination no fertilization membranes; 23 minutes after insemination some closely fitting fertilization membranes; 45 minutes after insemination 1 per cent 2's, others no development; 1 hour and 50 minutes after insemination about 2 per cent regular and irregular cleavage; 1 hour and 57 minutes after insemination, plate 7, figure 4; on the following morning most eggs had never segmented, rare plutei.

(2) 8 minutes after insemination no fertilization membranes; 24 minutes after insemination many eggs swollen and with rough surface; 45 minutes after insemination 33 per cent with surface highly modified, thickened and narrowly separated membrane; 1 hour and 53 minutes after insemination 50 per cent potato-like irregularity in form, plate 7, figure 5; on the following morning all were disintegrated.

(3) 5 minutes after insemination no separated membranes; 20 minutes after insemination many eggs swollen and with rough surface; 38 minutes after insemination 33 per cent with surface highly modified; 1 hour and 50 minutes after insemination 50 per cent potato-like irregularity in form; 1 hour and 57 minutes after insemination, plate 7, figure 6; on the following morning no development.

Phloxine Red

Phloxine red, Grübler, was used in 1:40,000 solution. No separated fertilization membranes were formed. Following both 10- and 15-minute irradiations there was ultimately complete cytolysis. The eggs swelled gradually, stained pink, then red, and formed blisters, some of the size of the egg.

The difference in effect between phloxine red and phloxine suggested that both be used on eggs from the same female. The results confirmed those on separate lots of eggs. Phloxine red in its action behaved somewhat like a basic dye, whereas the action of phloxine was clearly like that of an acid dye.

The following table gives an account of this investigation.

Summary of Results of Irradiation of Eggs in Solutions of Phloxine Red 1:40,000

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/15/39...........	(1)	28.6	1:40,000	0	Dark
	(2)	30.3	1:40,000	10	8633
	(3)	30.3	1:40,000	15	8633

Results. (1) No separated membranes 3 minutes after insemination, none pitted; 14 minutes after insemination pitting 1 per cent; 24 minutes after insemination eggs unchanged, no pitting evident; 39 minutes after insemination eggs unchanged; control beginning 2-cell 37 minutes after insemination; (1) eggs unchanged, no separated membranes 66 minutes after insemination; 3 hours and 26 minutes after insemination eggs unchanged, look normal; 6 hours after insemination 3 per cent cytolized; on the following morning 95 per cent disintegrated.

(2) 2 minutes after insemination no separated membranes, a few pitted; 11 minutes after insemination about 4 per cent blister cytolysis, cytoplasm stained uniformly pink, no vacuoles; 21 minutes after insemination 40 per cent stained and with blisters, 60 per cent not stained, have normal color but are enlarging; 28 minutes after insemination blister cytolysis, pink eggs increased to 75 per cent of total; 67 minutes after insemination 99 per cent pink, enlarged and in blister cytolysis, 1 per cent dark and enlarged, many with 1 or 2 blisters; 3 hours and 26 minutes after insemination eggs red with clear and stippled blisters; 6 hours and 6 minutes after insemination 100 per cent cytolized, red centers and pink blisters; on the following morning all disintegrated.

(3) 3 minutes after insemination no separated membranes; 4 minutes after insemination blister cytolysis beginning; 13 minutes after insemination 50 per cent pink with blisters, all the remaining 50 per cent swollen and some beginning to blister; 24 minutes after insemination all enlarged and pink, 90 per cent in blister cytolysis; 1 hour and 3 minutes after insemination all enlarged, pink, with one to many blisters; 3 hours and 21 minutes after insemination more eggs red with clear blisters, some the size of the egg, than with stippled blisters, much redder than in (2); 6 hours after insemination 100 per cent cytolized, red centers and pink blisters; on the following morning all disintegrated.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/17/39...........	(1)	28.8	1:40,000	0	Dark
	(2)	30.3	1:40,000	10	7172–7437–7703–7969
	(3)	30.0	1:40,000	15	7172–7437–7703–7969

Results. (1) 9 minutes after insemination no fertilization membranes; 17 minutes after insemination no membranes, surface of some eggs slightly rough; 22 minutes after insemination about 1 per cent with slightly roughened surface; 36 minutes after insemination eggs look normal, no fertilization membranes; 3 hours and 21 minutes after insemination no change; 4 hours and 19 minutes after insemination no change; on the following morning 95 per cent look like normal eggs, no blister cytolysis; control excellent young plutei. See plate 8, figure 6.

(2) 10 minutes after insemination no fertilization membranes, some pitting; 17 minutes after insemination no membranes, 1 per cent pitting; 22 minutes after insemination 50 per cent swollen and slightly pink, these cytolizing with blisters, darker eggs pitted and slightly swollen; 36 minutes after insemination 90 per cent swollen, 50 per cent stained and blistered; 3 hours and 22 minutes after insemination all swollen, 96 per cent red with blisters, 4 per cent dark and fragmented; 4 hours and 19 minutes after insemination same as 3 hours and 22 minutes but red eggs redder and blisters pink; on the following morning all cytolized, very red. See plate 8, figures 1, 4.

(3) 5 minutes after insemination swelling, staining, blistering; 12 minutes after insemination 100 per cent swollen, 95 per cent swollen and blistered; 18 minutes after insemination all swollen, pink, blistered; 31 minutes after insemination all swollen, stained, blistered; 3 hours and 19 minutes after insemination all red with blisters; 4 hours and 14 minutes after insemination all swollen, very red with pink blisters; on the following morning all cytolized, very red. See plate 8, figures 2, 3, 5.

Acridine

It is with especial interest that we take up the consideration of the photodynamic effect of acridine on *Lytechinus* eggs, since it was with this dye that Raab (1897–1898) discovered the phenomenon to which von Tappeiner (1906) gave the name photodynamic action.

In the present work acridine, Grübler, was used in concentrations from 1:20,000 to 1:640,000 in sea water. Even in the most dilute solutions few of the eggs formed well separated fertilization membranes, the germ nuclei did not unite, and there was no cleavage. During 20-minute irradiation of both the 1:20,000 and the 1:40,000 solutions the sky was overcast and the cultures got only 5 minutes sunlight. The eggs in nonirradiated 1:20,000 solutions that were exposed to diffuse light at a temperature of 29° C., and inseminated after 25 minutes, stained bright yellow and formed no fertilization membranes. Those in solutions irradiated and stirred for 20 minutes with the temperature held at 27.8° C. stained very yellow; many eggs burst, and no fertilization membranes were formed.

The eggs in the 1:40,000 solutions in diffuse light at 29° C. stained bright yellow, and fertilization membranes were formed on 12 per cent. Those in the 1:40,000 cultures, irradiated and stirred for 20 minutes with the temperature held at 28° C., stained bright yellow; fertilization membranes were not separated, but a bright protruding vesicle appeared, formed probably where the spermatozoon entered. It was only in the irradiated 1:40,000 culture that cytolysis appeared, occurring here to the extent of 20 per cent. It was clear that these solutions were much too strong, and the cultures were discarded.

In the work with 1:80,000 solutions of acridine, uninseminated eggs placed in the solutions of dye at a temperature of 29.3° C., and inseminated after 20 minutes, formed fertilization membranes. Uninseminated eggs placed in 1:80,000 solution, and irradiated and stirred for 20 minutes at a temperature of 29.2° C., formed no fertilization membranes. Because of poor sunlight both these cultures were discarded.

Uninseminated eggs in 1:160,000 cultures in diffuse light held at 29.3° C., inseminated after 20 minutes in the solution of the dye, formed fertilization membranes but underwent no further development. Uninseminated eggs irradiated and stirred for 20 minutes in 1:160,000 solutions held at 29° C., and inseminated after 20 minutes, likewise formed no fertilization membranes and failed to cleave. These cultures also were discarded because of poor eggs and poor sunlight, and the experiment was repeated on the day following.

Uninseminated eggs in the 1:160,000 cultures in diffuse light held at a temperature of 28.4° C., and inseminated after 20 minutes, formed excellent fertilization membranes, but the germ nuclei either failed to unite or united very slowly. There was a little abortive cleavage and no cytolysis, although some of the eggs were irregular. Uninseminated eggs irradiated and stirred for 20 minutes at a temperature of 28.4° C. in 1:160,000 solution, and inseminated at the end of 20 minutes, formed no fertilization membranes. The germ nuclei failed to unite in many eggs. Blister cytolysis appeared about 50 minutes after insemination and gradually increased during the ensuing hour and a half until it involved 50 per cent of the eggs. Clear vesicles were then breaking away from the eggs. There was no cleavage.

It was evident from this experiment that the 1:160,000 solution was toxic even in the diffuse-light cultures. A concentration of 1:320,000 was then tried. Uninseminated eggs kept in a 1:320,000 culture in diffuse light at a temperature of 28.4° C., and inseminated after 20 minutes, formed excellent fertilization membranes. The germ nuclei were slow in uniting. After 55 minutes these eggs began to divide badly to 2 cells. The insemination-cleavage interval in the sea-water control was 42 minutes. In the experimental culture 90 per cent of the eggs underwent irregular cleavage; 10 per cent remained spherical and undivided. There was no cytolysis. Uninseminated eggs irradiated and stirred for 20 minutes in 1:320,000 solution at a temperature of 28.3° C. formed no fertilization membranes. In 55 minutes about 15 per cent of these eggs showed blister cytolysis. Many eggs showed two or more nuclei. Within half an hour blister cytolysis had increased to 30 per cent. There was no cleavage.

Even at this concentration, 1:320,000, the toxicity of acridine in the diffuse-light culture was evident. Therefore the remaining experiments were performed with 1:640,000 solution. Uninseminated eggs kept in 1:640,000 solution of acridine at a temperature of 28.8° C. for 10 minutes and then inseminated formed excellent fertilization membranes, cleaved to irregular 2-cell and irregular 4-cell stages, and continued their cleavage abnormally. Cytolysis did not appear. Uninseminated eggs irradiated in 1:640,000 solution of acridine for 10 minutes at a temperature of 28.3° C. and then inseminated rarely formed well separated fertilization membranes, although nearly all had tight membranes, separated just enough to be seen. The nucleus in these eggs remained intact and the surface of some eggs became roughened, some even showing blistering. Finally there were many eggs with clear protrusions from the surface. There was no cleavage. Uninseminated eggs irradiated and stirred for 20 minutes at a temperature of 28.9° C. formed no fertilization membranes. The surface of the eggs was slightly roughened, the nuclei remained intact, and the cytoplasm became slightly granular; there was rare cytolysis and no cleavage. Uninseminated eggs irradiated and stirred for 20 minutes at a temperature of 31.7° C., and then inseminated, formed no fertilization membranes. In 35 minutes 10 per cent of the eggs showed blister cytolysis which gradually increased in amount, the nuclei remaining intact and somewhat swollen. Many of the blister-cytolized eggs disintegrated, clear vesicles breaking from the eggs and floating about in the solution. There was no cleavage.

In these cultures all the eggs were stained slightly yellow. Further, in all except the control there were two nuclei, one large and one small; that is, the egg and sperm nuclei had failed to unite.

Results of the Study of Sections of Eggs from Acridine Cultures

In the nonirradiated acridine cultures (1:640,000) the eggs developed with centrosomal caps but without or with greatly reduced asters. The chromosomes divided with some lagging (fig. 35). Division of the cytosome was poor. It was evident that the dye had some toxic effect.

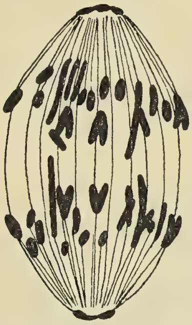


FIG. 35. Acridine, 7/25/37, 3:43 P.M.

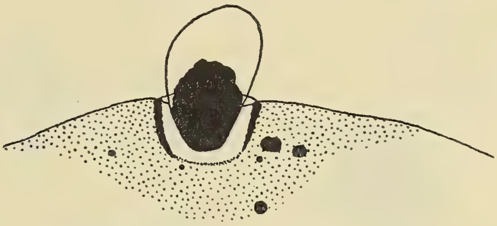


FIG. 36. Acridine, 7/25/37, 5:25 P.M.

In the cultures that were irradiated in the 1:640,000 solution of acridine in sea water and then inseminated, development was uniformly bad. The sperm nuclei enlarged, some of them even before entering the egg (fig. 36). After entering the egg they always enlarged. All the figures shown were drawn from sections of eggs that had been irradiated 10 minutes and cooled to 28.3° C. during irradiation. The characteristic feature of development in these was the failure of the female and male nuclei to unite. From one to seven male nuclei entered the egg and enlarged greatly. One male nucleus might fuse with the egg nucleus, which then proceeded through division without division of the cyto-

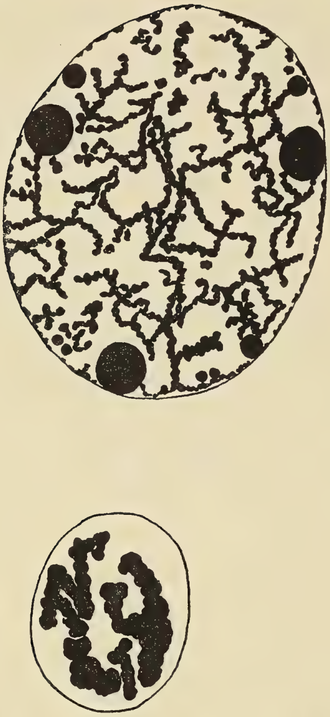


Fig. 37. Acridine, 7/25/37, 5:25 p.m.

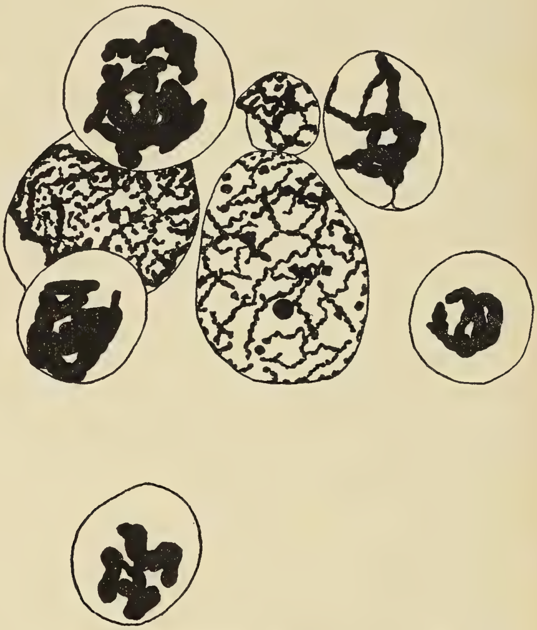


Fig. 38. Acridine, 7/25/37, 5:25 p.m.

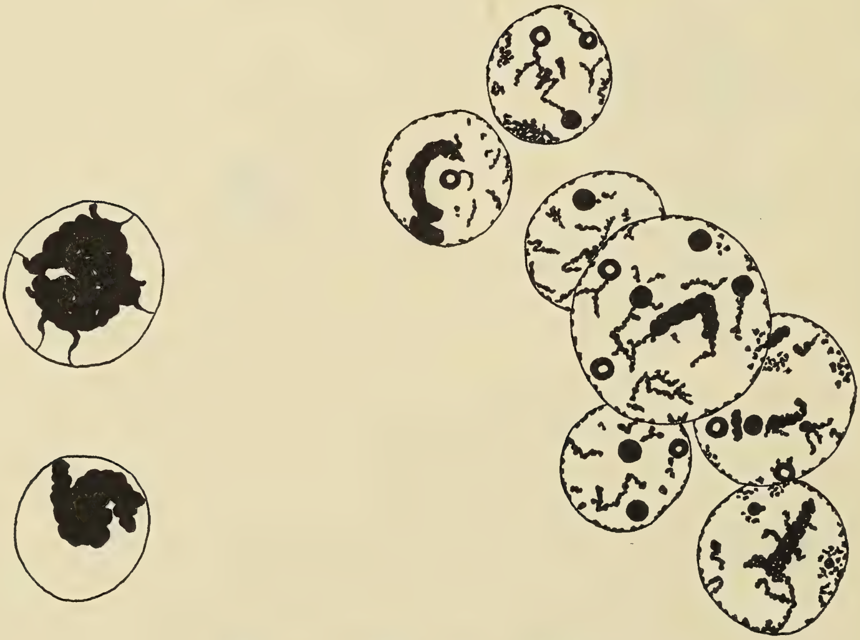


Fig. 39. Acridine, 7/25/37, 5:25 p.m.

some (figs. 37, 38, 39). The supernumerary sperm nuclei degenerated in place in the cytosome without division. The fertilized egg nucleus divided irregularly by numerous constrictions to form a group of nuclear vesicles (figs. 39, 40).

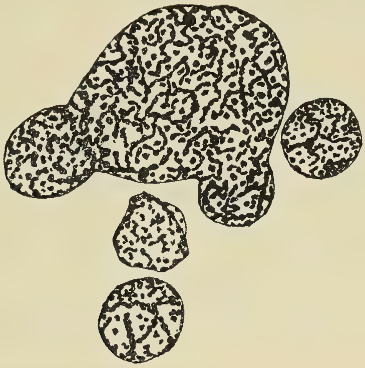


FIG. 40. Acridine, 7/25/37, 5:25 P.M.

THIOINDIGO RED

Thioindigo red B, Kahlbaum, was used in solutions of somewhat less than 1:25,000. The dye showed little photodynamic effect. In irradiated eggs the fertilization membrane was not well separated, nevertheless cleavage and development to the pluteus stage was normal.

The following table gives the results of the investigation.

Summary of Results of Irradiation of Eggs in Solutions of Thioindigo Red 1:25,000

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/2/39.............	(1)	28.8	1:25,000	0	Dark
	(2)	29.8	1:25,000	10	9562
	(3)	30	1:25,000	15	9562

Results. (1) 2 minutes after insemination membranes well separated; 28 minutes after insemination surface of all eggs smooth, membrane well separated; control, first constriction beginning 38 minutes after insemination; (1) no constrictions; (1) in and into 2's 41 minutes after insemination; control, 4's 59 minutes after insemination; (1) 4's 61 minutes after insemination; swimming blastulae 5½ hours after insemination; most are excellent plutei with long arms at 8 A.M. on following morning.

(2) 2 minutes after insemination well separated membranes; 30 minutes after insemination surface of all eggs smooth, membrane well separated; control first constrictions 38 minutes after insemination; (2) many in 2's 39 minutes after insemination; most in 4's 59 minutes after insemination; swimming blastulae 5½ hours after insemination, some irregular cleavage; on following morning most are excellent plutei with long arms.

(3) Fertilization membranes irregularly separated 4 minutes after insemination, eggs slightly irregular in outline; surface of all eggs slightly rough 5 minutes after insemination, membrane not well separated; deep constriction and into 2's 37 minutes after insemination; 2's, 4's 59 minutes after insemination; 4's and 8's 79 minutes after insemination; swimming blastulae 5½ hours after insemination; on following morning most were living; most plutei with short arms, some with long arms.

PHLOROGLUCIN-TRYPAN RED

Phloroglucin-trypan red was used in 1:40,000 and 1:80,000 solutions. In 1:40,000 solutions fertilization membranes were not separated in either the nonirradiated or the irradiated cultures. In the 1:80,000 solutions there was rare separation of fertilization membranes, about 1 per cent in nonirradiated dye solutions, but fewer following 15-minute irradiation (1 egg in several hundred). Cleavage took place in 3 per cent of the nonirradiated eggs and was exceedingly rare in irradiated eggs.

The following table gives in detail the results of the investigation.

Summary of Results of Irradiation of Eggs in Solutions of Phloroglucin-Trypan Red 1:40,000 and 1:80,000 for Various Periods of Time

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/12/39............	(1)	29.0	1:40,000	0	Dark
	(2)	28.9	1:40,000	10	1328–1769–1870
	(3)	28.9	1:40,000	15	1328–1769–1870

Results. (1) 4 minutes after insemination no fertilization membranes; 9 minutes after insemination sperms active but no separated membranes; 17 minutes after insemination less than 1 per cent pitted; 31 minutes after insemination same as at 17 minutes; 43 minutes after insemination none yet divided; 64 minutes after insemination unstained, unchanged; 4 hours after insemination no development, egg has normal form; on the following morning most eggs misshapen and sticking together.

(2) 6 minutes after insemination no fertilization membranes; 10 minutes after insemination 1 per cent pitted; 13 minutes after insemination not more than 1 per cent pitted; 31 minutes after insemination same as at 13 minutes; 44 minutes after insemination none divided; 61 minutes after insemination unstained, unchanged; 4 hours after insemination no development, egg has normal form; on the following morning eggs adhering to one another, most are slightly disintegrated.

(3) 2 minutes after insemination no fertilization membranes; 6 minutes after insemination no pitting; 8 minutes after insemination none pitted; 26 minutes after insemination same as at 8 minutes; 39 minutes after insemination none divided, 1 per cent pitting; 58 minutes after insemination unstained, unchanged; 4 hours after insemination no development, egg has normal form; on the following morning eggs adhering to one another, most are slightly disintegrated.

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/14/39...........	(1)	27.6	1:40,000	0	Dark
	(2)	29.7	1:40,000	10	3984–9297
	(3)	29.9	1:40,000	15	3984–9297
	(4)	29.9	1:80,000	15	3984–9297
	(5)	27.6	1:80,000	0	Dark

Results. (1) 7 minutes after insemination no separated membranes; 17 minutes after insemination no separated membranes; 1 hour and 25 minutes after insemination no change; 3 hours and 15 minutes after insemination all eggs intact; 5 hours and 50 minutes after insemination eggs intact, no separated membranes; on the following morning all eggs cytolized, some masses stained red either in whole or in part.

(2) 8 minutes after insemination no separated membranes; 18 minutes after insemination no separated membranes and little pitting; 1 hour and 25 minutes after insemination no change; 3 hours and 16 minutes after insemination all eggs intact; 5 hours and 9 minutes after insemination eggs intact, no separated membranes; next morning eggs same as in (1).

(3) 4 minutes after insemination no separated membranes; 14 minutes after insemination no separated membranes; 1 hour and 20 minutes after insemination no change; 3 hours and 12 minutes after insemination all eggs intact; 5 hours and 53 minutes after insemination eggs intact, no separated membranes; on the following morning same as (1) and (2).

(4) 6 minutes after insemination no separated membranes; 15 minutes after insemination 1 egg in several hundred has a separated membrane; 1 hour and 25 minutes after insemination 20 per cent pitted, exceedingly rare 8-cell; 3¼ hours after insemination pitting not increased, rare hollow spheres; 5 hours and 51 minutes after insemination eggs intact, no separated membranes; on the following morning like (1), (2), and (3).

(5) 5 minutes after insemination 1 per cent well separated membranes; 14 minutes after insemination 1 per cent well separated membranes; 1 hour and 24 minutes after insemination 3 per cent in 4's and 8's, others unchanged; 3 hours and 14 minutes after insemination hollow spheres; 5 hours and 50 minutes after insemination eggs intact, no separated membranes; on the following morning like (1), (2), (3), and (4), but fewer stained.

MADDER EXTRACT

Madder extract, Pickhart and Kuttrof, was only slightly soluble. It produced no effect in either irradiated or nonirradiated solutions. See table below.

Summary of Results of Irradiation of Eggs in Saturated[1] Solutions of Madder Extract

Date	Culture no.	Temperature (°C.)	Time (min.)	Intensity of sunlight (foot-candles)
7/23/39..........................	(1)	29.0	0	Dark
	(2)	30.6	20	7969

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[1] Dry extract made up 1:20,000 in sea water was not completely dissolved; the saturated solution so obtained was used.

Results. (1) 5 minutes after insemination strong, well separated membranes; 45 minutes after insemination, into 2's; 1 hour and 52 minutes after insemination 4's, 8's, 12's.

(2) 5 minutes after insemination delicate but well separated membranes; 45 minutes after insemination, into 2's; 1 hour and 52 minutes after insemination excellent 4's and 8's.

PURPURIN-MADDER

Purpurin-madder, Strohmeyer and Lauth, was very slightly soluble. It produced no photodynamic effect.

OXAMINE RED

Oxamine red B. N., Badische, was used in 1:40,000 solution. In both non-irradiated and irradiated solutions only about 5 per cent of the eggs formed separated fertilization membranes. The irradiated eggs formed lobular blebs, a solid core with a cortex of small vesicles of uniform size finally resulting.

The results of the investigation are shown in the following table.

Summary of Results of Irradiation of Eggs in Solutions of Oxamine Red 1:40,000

Date	Culture no.	Temperature (°C.)	Concentration	Time (min.)	Intensity of sunlight (foot-candles)
7/22/39...........	(1)	28.0	1:40,000	0	Dark
	(2)	30.9	1:40,000	20	8633

Results. (1) 4 minutes after insemination 1 per cent with membranes; 11 minutes after insemination 5 per cent with membranes separated; 31 minutes after insemination no blebs.

(2) 5 minutes after insemination 1 per cent with membranes; 11 minutes after insemination 5 per cent with membranes separated; 28 minutes after insemination 12 per cent lobular blebs; 33 minutes after insemination solid core with cortex of small uniform-sized vesicles.

GLUTATHIONE, CYSTEINE, AND CYSTINE

Lytechinus eggs were inseminated at 10:02 A.M. At 10:10 they were placed in the following solutions:

Glutathione:	Cysteine:	Cystine:
(1) 1:3000	(2) 1:3000	(3) 1:3000
(4) 1:6000	(5) 1:6000	(6) 1:6000
(7) 1:9000	(8) 1:9000	(9) 1:9000
Control in sea water		

The study of these eggs was begun at 11:15 and continued intensively throughout the day. The surface of the eggs in glutathione solutions, (1), (4), and (7), was rough and slightly irregular from the first, whereas the eggs in the cysteine solutions, (2), (5), and (8), and in the cystine solutions, (3), (6), and (9), were smooth and regular.

Like the eggs in the control, the eggs in all the solutions were entering the 2-cell stage at 10:40, 38 minutes after insemination, those in the glutathione solutions, (1), (4), and (7), showing greater variation than those in the cysteine and the cystine solutions. At 12:00 the eggs in (9) were slightly in advance of the others; the control culture, for example, showed no eggs beyond the 16-cell stage, whereas (9) showed many, also the control culture showed many eggs below the 12-cell stage and (9) showed few.

At mid-afternoon the eggs in the cysteine solutions, (2), (5), and (8), had become abnormal, only a few becoming rotating blastulae, and few leaving the bottom of the culture dish. At 4:10, for example, in (7) all were off the bottom, in (8) most were still on the bottom, and in (9) all were off the bottom.

At 8:00 on the following morning, the eggs in glutathione, (1), (4), and (7), showed (1) dead, late gastrulae; (4) live plutei; (7) live plutei. Those in cysteine, (2), (5), and (8), were all dead, none having developed beyond blastulae. Those in cystine, (3), (6), and (9), showed (3) late gastrulae, (6) plutei, broad, and (9) excellent plutei. The control showed excellent plutei.

Results of the Study of Sections of Eggs from Glutathione, Cysteine, and Cystine Cultures

In the control culture in sea water most chromosomes were very sharply stained.

Most of the eggs from the glutathione culture showed nucleoli and irregular chromosomes on the spindle, although there were some eggs in which no nucleoli were evident.

In the eggs from the cysteine culture the mitotic figure was normal in appearance, but nucleoli and irregular chromosomes or irregular segments of the chromatin net were present on the spindle.

In the eggs from the cystine culture the chromosomes were irregular and there were many nucleolus-like bodies on the spindle.

There was nothing in the appearance of the mitotic figures that suggested any reason for the differences in later behavior.

DIIODO-TYROSINE

Several days were devoted to a study of the effect of diiodo-tyrosine, the product of Hoffman-La Roche, Inc., being used. The results were as follows.

Lytechinus eggs were transferred 5 minutes after insemination to the following solutions in sea water:

(1) 1:2000
(2) 1:3000
(3) 1:5000
(4) 1:8000
(5) 1:10,000

In (1) 3 minutes later the fertilization membrane was close to the egg; many eggs were cytolizing. In (2) 5 minutes after transfer the fertilization membrane was slightly separated from the egg. There was no cytolysis. The fertilization membrane was well separated in (3), (4), and (5) and there was no cytolysis. Ten minutes after transfer, in (1) the fertilization membrane was closely in contact with the egg, and (2), (3), (4), and (5) were the same as earlier, (2) with a slightly separated fertilization membrane and (3), (4), and (5) with a well separated fertilization membrane. The close contact of the fertilization membrane in (1) seemed to be caused by lessening of surface tension of the egg. The diameter of (1)'s was greater than that of the other eggs. The diameter of (5)'s was the least in this set.

The eggs in (1) 30 minutes after insemination were very soft; many were cytolized; the nucleus was intact. Those in (2) and (3) were somewhat enlarged, with an intact nucleus. Those in (4) and (5) had a smaller diameter and an intact nucleus.

It was found that fertilization was incomplete in all these eggs, the male and female nuclei never having united.

Another lot of eggs was inseminated and 8 minutes later samples of these eggs were dropped into the cultures just described. In (1) the eggs cytolized. In (2), (3), (4), and (5) cleavage of the eggs began 38 minutes after insemination, in tempo with the control. In later cleavage (3), (4), and (5) were better than (2).

In another experiment the stock solution from which all cultures were made was a 1:1000 solution in distilled water. Eggs were transferred 8 minutes after insemination to the following solutions in sea water:

(1) 1:5000
(2) 1:6000
(3) 1:7000
(4) 1:8000
(5) 1:9000

Ten minutes later the eggs in all these cultures were in prophase of division, as were those of the control. The eggs in the experimental cultures 42 minutes after insemination were slightly slower in their development than those in the control, which were in mid anaphase. The eggs in the control 49 minutes after insemination either had divided or were in late anaphase. The control was ahead of (5), which was the most advanced of the experimental series. Two hours after insemination, when the eggs of the control had reached the 12- and 16-cell stage and those in (5) were in the same stage, those in (4) showed rare 12-cell stages and all the eggs in (3), (2), and (1) were still in the 8-cell stage. On the following morning at 8 o'clock (5), (4), (3), (2), and (1) all contained normal young plutei with skeletal rods, normal prism larvae with enterocoeles, and late gastrulae with enterocoeles forming. Development continued normally, and in the afternoon all had reached a fine normal pluteus stage. Those in (1) were slightly opaque.

In the last experiment, *Lytechinus* eggs were transferred 7 minutes after insemination to the following solutions in sea water:

(1) 1:5000
(2) 1:6000
(3) 1:7000
(4) 1:8000
(5) 1:9000

All the eggs looked alike and in good shape 10 minutes later, and all cleaved regularly through 2's, 4's, 8's, etc., to the 64-cell stage. On the following morning the eggs of the control had developed to normal young plutei. All the eggs of (1) through (5) had developed to gastrulae, which never escaped from the fertilization membrane, but died in this stage.

HEPARIN

The Nature of the Fertilization Membrane

Is the fertilization membrane simply lifted from the surface of the egg at fertilization, or is it in the nature of a precipitation or a coagulation membrane formed during the fertilization reaction? Four experiments were performed for the purpose of answering these questions.

Lytechinus eggs were inseminated in a 1:500,000 solution of heparin in sea water. A strong normal fertilization membrane was formed. Other eggs from the same female were inseminated in a 1:100,000 solution of heparin. The result was a barely distinguishable membrane, irregularly separated from the egg. The membranes in the eggs in the controls in sea water were strong and well separated in both instances.

Uninseminated *Lytechinus* eggs were placed in 1:50,000 solution of heparin in sea water and inseminated at once. The eggs in the control in sea water all showed well separated fertilization membranes; the eggs in heparin solution showed very poorly separated membranes. Division was the same in both cultures.

Uninseminated *Lytechinus* eggs were placed in 1:50,000 solution of heparin in sea water and inseminated 7 minutes after being placed in this solution. The control showed well separated fertilization membranes. The eggs in the solution of heparin showed weak and poorly separated membranes. The membranes in the eggs in 1:50,000 solution of heparin were never well separated.

SUMMARY AND DISCUSSION

Transmission of visible light by some of the filters was so low that the intensity of the light transmitted was not sufficient to produce photodynamic effect. With dyes that produced a photodynamic effect, irradiation of a solution of the dye resulted in the formation of a photocompound. This photocompound was the active agent in the production of the photodynamic effect.

In neutral red, aging of the pre-irradiated solution resulted in loss of the effect that was produced by irradiation.

There was a progressive increase in effect of 5-, 10-, and 15-minute irradiation.

Reduction of the intensity of irradiation resulted in reduction of the photodynamic effect.

Continued irradiation may result in the inhibition of blister cytolysis. This effect seemed to be due to the coagulation of the surface of the egg.

The threshold for violent surface reaction (blister cytolysis) of *Lytechinus* eggs in 1:150,000 solution of neutral red in sea water lay at about 2500 foot-candles. From this point to about 4300 foot-candles violent surface reaction usually stood at about 2 per cent. Between 7000 and 7500 foot-candles it increased to 20–25 per cent, and between 8000 and 9500 foot-candles it increased to 75–90 per cent. At intensities from 3000 to 10,000 foot-candles there was a regular increase in the violence of the surface reaction and complete inhibition of the cleavage processes.

Corning glass filters nos. 428 and 430, which remove the noninjurious red but transmit the blue, gave a high percentage of blister cytolysis at a low intensity in foot-candles.

Irradiation of unstained eggs with subjection to a temperature of 34.8° C. did not result in an irreversible injury to the eggs.

Irradiation in some of the solutions of dye at temperatures above 32° C. resulted in injury from which the eggs did not recover.

Von Tappeiner (1906) showed that, in addition to the photodynamic effect of neutral red, a 1:40,000 solution of this dye caused death even in the dark. This statement cannot be made for the 1:150,000 solution. Politzer (1934) asks whether injuries to the cell produced by neutral red are caused by mechanical injury through accumulated dyestuff, poisonous action of neutral red, or photodynamic action of neutral red. In the present work with the basic dye neutral red, two effects were noted: (1) a superficial effect which might result ultimately in complete blister cytolysis, and (2) an effect involving the chromosomes.

In blister cytolysis the formation of blisters starts at a single point. Adjacent blisters come into contact with one another until the entire surface is covered. The content of these blisters is liquid, is clear on the living egg, and in the fixed egg seems to be the same as the cytoplasm of the egg with all formed components removed. It is conceivable that these components could be filtered out, but there is no evidence of the accumulation of granules at the point where the cytoplasm might have been extruded.

Irradiation of 5 minutes in 1:150,000 solution of neutral red produced lagging of chromosomes. When the irradiation was continued longer, the effects produced were more striking in that there was more definite lagging of the chromosomes and pycnosis during division. Pseudoamitotic figures were common. Lagging is closely associated with an increase in intensity of irradiation and an increase in temperature.

There is a close resemblance between this effect and that produced by Roentgen rays. Politzer (1934) has described the Roentgen influence as producing a primary effect marked by pycnosis and pseudoamitosis, a mitosis-free interval, and a secondary effect marked by rhexis and deflection of chromosomes and part nuclear formation. The same statement may be made concerning the influence of neutral red, depending on the length and intensity of irradiation.

The experiments of the present paper show that at intensities from 3000 up to 10,000 foot-candles there is a regular increase in the violence of the surface reaction and complete inhibition of the cleavage processes. With Corning glass filters which removed the noninjurious red light but transmitted the blue, the results were striking.

Early in this work it became evident that solutions of Janus green B undergo deterioration quickly. In order to get consistent results from day to day, freshly prepared solutions must be used. Solutions of Janus green were found to be highly toxic. The toxicity of the dye was increased by irradiation in sunlight. The chromosomes showed irregularity in form and persistent lagging in distribution.

The formation of vacuoles with inclusions in eggs treated with solutions of thionin and irradiated for 20 minutes is a result that one expects to see with an acid dye, and its occurrence with a basic dye is surprising. The enlargement of the male nucleus shown in sections is of interest.

The fact that there is great variation in the reaction of different lots of eggs stained in brilliant cresyl blue was emphasized by the behavior of eggs in the nonirradiated control kept in darkness, 25 per cent of the eggs in one experiment blistering. In the other nonirradiated dye controls blistering varied from 1 to 4 per cent. There was nothing in the results of the experimental work indicating an intensity threshold at which cytolysis might be expected.

Although neutral red and brilliant cresyl blue stain the same components, the effects of staining with brilliant cresyl blue differ widely from those following staining with neutral red. A perfect but shortened achromatic figure was characteristic of eggs stained with brilliant cresyl blue, but so far as chromosomes are concerned no perfect mitotic figures were found. The chromosomes were usually very much bent.

Politzer (1925) found that brilliant cresyl blue and auramin checked the course of cell division at two stages, namely, between the resting stage and spireme, and between the asters and metakinesis. Becker (1932) found that methylene blue, toluidine blue, thionin, and cresyl blue caused greater injury to karyokinesis than did neutral red, Janus green, neutral violet, safranin, and phenosafranin. Politzer's (1925) work dealt with the toxic influence of the dye, which was used in solutions stronger than the 1:80,000 solutions described in this paper.

Following the use of cresyl violet, the most common type of abnormality was pycnosis. The next most common abnormality was lagging of the chromosomes, although polyspermy was also common. In many cases the male and female nuclei failed to unite.

Safranin O was slightly toxic in diffuse-light cultures, and irregular cleavage and cytolysis were produced by irradiation.

The effect of irradiation in Magdala red echt is somewhat similar to that in solutions of neutral red, the difference being that with Magdala red echt the eggs swell before producing clear spherical blisters.

In nonirradiated eggs in 1:20,000 solution of nigrosin there was slight lagging of chromosomes during their approach to the poles, and there were no globules in the cytoplasm. Irradiation in 1:20,000 solution of nigrosin resulted in the production of large, deeply stained globules in the interzonal region.

Auramin O was exceedingly toxic. The primary effect on the eggs was shown by the achromatic figure, which appeared as a short anastral figure, little longer than the diameter of a normal nucleus. In the irradiated cultures the central spindle had no asters or astral rays. There was very little cell division. There was some division of the nuclei without cytoplasmic division.

Ludford (1936) used auramin O in 1:20,000 solution on a culture of carcinoma 63 and found arrest of mitosis at metaphase. He notes that with higher concentrations of auramin mitosis may stop at this stage. The solutions used in the present work were much more dilute and caused essentially the same effects. Uninseminated eggs irradiated in auramin O solutions and fertilized after removal to diffuse light showed the same reactions as eggs that were not irradiated until after insemination. The effect of increased temperature is of interest here. At 28° C. eggs in nonirradiated diffuse-light cultures divided normally; those irradiated for 10 minutes showed no cytolysis and gave nuclear division but no division of the cytoplasm; those irradiated for 20 minutes showed rare cytolysis but were otherwise like eggs of the 10-minute irradiation; and eggs inseminated at 28° C. and then allowed to come to just below 32° C. during a 20-minute irradiation showed much fine blistering shortly after insemination, cytolysis increasing and disintegration following during the next 20 minutes.

Brilliant green was used in concentrations of 1:50,000 to 1:1,600,000. The treatments have revealed different degrees of cytolysis which if taken separately would merit a special classification. The lowest degree seems to lie in blister cytolysis, which may be of smaller or larger extent; beyond that, disintegration of the egg may occur. The next degree of injury seems to result in extreme irregularity of the egg with the formation of lobes and lobules, with or without the formation of blisters. A further degree of injury results in colorless cytolysis, the color of the dye fading and the egg breaking into innumerable fine colorless blisters. Finally, so great an injury may result that the egg remains spherical, its surface coagulated so quickly that no superficial reaction is possible.

Colorless cytolysis often occurs. It is possibly to be interpreted as oxidation of the cellular constituents with reduction of the dye.

The work with pyronin emphasized the fact that irradiation in 1:25,000 solution of dye killed the eggs before blister cytolysis could take place, this effect resembling that with irradiation in brilliant green. With irradiation after treatment with 1:150,000 solution of pyronin in sea water there was a high degree of blister cytolysis. The eggs showed a definite increase in the effect of the duration of irradiation; for example, after 15 minutes irradiation eggs showed pseudoamitosis and nuclear fragmentation, and after 20 minutes irradiation there was failure of the nuclei to unite.

The experiments with rhodamine B were done with 1:25,000 solution in sea water. In nonirradiated eggs the mitotic figure was normal and the chromosomes divided regularly. With eggs irradiated in solution of rhodamine B there was considerable variation in the alignment of chromosomes and there was lagging in the distribution of chromosomes.

The experiments with fluorescein were of two kinds, those done with pre-inseminated and those with post-inseminated eggs. Inseminated eggs transferred to solutions varying from 1:150,000 to 1:10,000 developed perfectly in both irradiated and nonirradiated solutions. All the experiments with uninseminated eggs were done with 1:50,000 solution of fluorescein in sea water. In these experiments uninseminated *Lytechinus* eggs were transferred to 1:50,000 solution of fluorescein in sea water in diffuse light. The eggs in this culture began to divide 42 minutes after insemination and proceeded regularly to normal blastulae, gastrulae, and plutei. Uninseminated eggs from the same stock were transferred to 1:50,000 solution of fluorescein in sea water, irradiated for 20 minutes, and then inseminated. No separated fertilization membranes were formed. All the eggs were indented or pitted about 48 minutes after insemination. Aberrant development followed. If the cultures were not irradiated, normal development took place in the solutions of fluorescein, but if the cultures were irradiated with sunlight abnormal development followed.

Blum (1930*a*, 1930*b*) conducted experiments under carefully controlled conditions with fluorescein, eosin, and erythrosin. He demonstrated that previously irradiated solutions of these dyes bring about hemolysis in concentrations at which nonirradiated solutions do not. Some bleaching of the dye takes place on irradiation, yet hemolysis is not due to the products of bleaching, as completely bleached solutions will not cause hemolysis. Nonirradiated eosin and erythrosin will produce hemolysis, but only in sufficiently high concentrations. Blum produces evidence that oxidation is a factor in photodynamic hemolysis. He concluded that oxidation must be considered as a probable cause of photodynamic hemolysis and all other photodynamic phenomena. His findings render untenable the sensitization theory of von Tappeiner and other theories that require the simultaneous action of light and photodynamic substances.

With eosin Y essentially the same facts were found. With fertilized eggs, even when the solutions in sea water were neutralized the experiments were never satisfactory because the eggs seemed protected by the fertilization membrane. With unfertilized eggs, however, consistent results were obtained with both 1:75,000 and 1:150,000 solutions in sea water. In diffuse-light cultures a strong, well separated fertilization membrane developed after insemination and the eggs kept pace with those of the sea-water control throughout cleavage. In irradiated cultures inseminated after irradiation, although fertilization occurred, the fertilization membrane was not lifted from contact with the egg. In all the irradiated cultures some blistering appeared within 15 minutes after insemination. The striking feature, however, was pitting of the surface of the egg, followed by the formation of numerous pink vacuoles containing refringent granules. In all except the blistered eggs, which became spherical masses of small globules, mitotic figures appeared, and the vacuolated eggs completed compact and irregular cleavage. At the end of 5½ hours, when both the sea-water control and the diffuse-light eosin Y control had reached the normal swimming-blastula stage, the irradiated cultures were increasingly vacuolated and cytolizing, depending on the duration of irradiation and the temperature to which the cultures had been subjected.

The experiments with phloxine gave striking results. Uninseminated eggs were irradiated and inseminated at the end of irradiation. Within 7 minutes after the end of 15-minute irradiation, 10 per cent of the eggs were pitted and vacuolated. Vacuolization increased to 50 per cent during the next half-hour. During this time most of these eggs formed blisters of the neutral red type. Finally all the irradiated eggs disintegrated.

Menke (1935*a*) used solutions of phloxine in his study of the photodynamic action of normal and malignant cells in vitro. He found that immediately after irradiation the affected cells began to contract, cell inclusions began a rearrangement, and there was a progressive change from a more or less definite arrangement of fat globules and other inclusions toward a chaotic distribution of these particles. The optical density of the cell increased, the cytolytic process began, and many small blebs were formed over the entire surface of the cell membrane. These blebs were not filled with a clear fluid, but seemed to contain undiluted cytoplasm of the same appearance and optical density as that which was in the main body of the cell. In many instances these blebs were observed to separate from the main body of the cell and wander some distance away. Menke's experiments differed from those described in the present paper in that the dye was not absorbed by the cells in the plasma media until after irradiation had produced its effect. In the present experiments the cells became vacuolated before blister cytolysis, which may be regarded as the same as formation of blebs, occurred. Attention has been called earlier to the fact that the content of the blisters noted in the present paper is the same as the cytoplasm with the formed components removed.

Phloxine red in its action behaved somewhat like a basic dye, whereas the action of phloxine was clearly that of an acid dye.

The acridine used in this work was highly toxic. In nonirradiated cultures in 1:640,000 solution the chromosomes divided with some lagging and division was poor. In the irradiated cultures of the same concentration the sperm nuclei enlarged and usually failed to unite with the egg nucleus, which divided irregularly by constrictions to form a group of nuclear vesicles. The photodynamic effect of the dye was clearly increased by the slightest increase of intensity of light. The difference between the effect of rearing the eggs in darkness and in diffuse light was evident.

Eggs placed in solutions of the acid dyes Congo red, fluorescein, eosin, and phloxine behaved in the same way. They all became vacuolated. Reactions of the chromosomes followed vacuolization. In solutions of the acid dyes Martius yellow and alizarin red S, no photodynamic effect occurred.

In solutions of the basic dyes, formed components in the eggs were stained, but in thionin vacuoles were formed, and in Magdala red and pyronin the eggs were indented or pitted. In auramin O and in acridine the cytoplasm stained but formed components were not stained.

The work described in this paper is in a field somewhat similar to that cultivated by H. F. Blum and his associates (1930 to 1940). Blum has worked with hemolysis following irradiation with the acid dyes fluorescein, eosin, erythrosin, and particularly rose bengal. The most detailed part of the present paper deals with the effect, cytolysis, of a basic dye, neutral red, on the transparent egg of a sea urchin. Blum and Hyman (1939*b*) discuss the effect of concentration of dye on hemolysis time. Some differences in effect between an acid dye, which acts on the membrane, and a basic dye, which stains formed components of the cytoplasm, must be expected, and probably the differences between the work of Blum and Hyman and that of the present study can be ascribed to this fact. In the experiments reported in their last two papers Blum and Gilbert (1940*a*, 1940*b*) have irradiated their material with monochromatic light. In the present study filters have been used, but irradiation in general was with mixed light. Blum and Hyman (1939*a*) found a rather close agreement with the Bunsen-Roscoe reciprocity law. Blum and Gilbert (1940*a*), using monochromatic light, found that apparently a cell may receive sufficient damage to lyse but may retain its structure for a long time before it breaks up. No sharp threshold exists. The data obtained show that the deviation from the reciprocity law reported by Blum and Hyman was due neither to inaccurate energy measurements nor to the use of mixed light as against the monochromatic irradiation in the present (1940*a*) instance. The fundamental photochemical reaction obeys the reciprocity law rigidly, and deviations from it such as were obtained by Blum and Hyman and by Lillie, Hinrichs, and Kosman (1935) are due to the "biological" part of the reaction. The work of the present paper shows a threshold for blister cytolysis and a close adherence to the reciprocity law. Blum and Gilbert (1940*b*) discuss the quantum requirements for photodynamic hemolysis. In the present paper no such study has been made. The results of the photodynamic effects of neutral red on mitosis resemble those described by Kemp and Juul (1932) as following irradiation in tissue cultures with ultraviolet rays.

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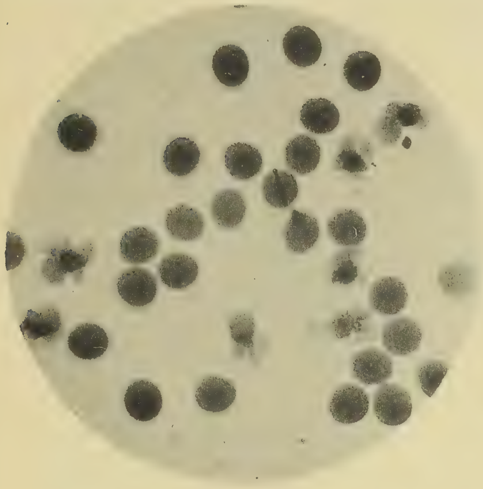
PLATES

Photographs of living eggs, inseminated before irradiation in solutions of dye. Plate 5, figures 2 and 6 taken with 4-millimeter objective and 10× ocular; all others taken with 16-millimeter objective and 10× ocular. Argus camera, Eastman 35-millimeter Panatomic X and Super X film.

PLATE 1

Neutral Red 1:150,000 (see text page 29)

FIG. 1. Grübler lot II. 15-minute irradiation. 30 minutes after insemination.
FIG. 2. Grübler lot I. 15-minute irradiation. 29 minutes after insemination.
FIG. 3. Grübler lot I. 15-minute irradiation. 33 minutes after insemination.
FIG. 4. Grübler lot I. 10-minute irradiation. 41 minutes after insemination.
FIG. 5. Grübler lot I. 10-minute irradiation. 42 minutes after insemination.
FIG. 6. Control. 44 minutes after insemination.



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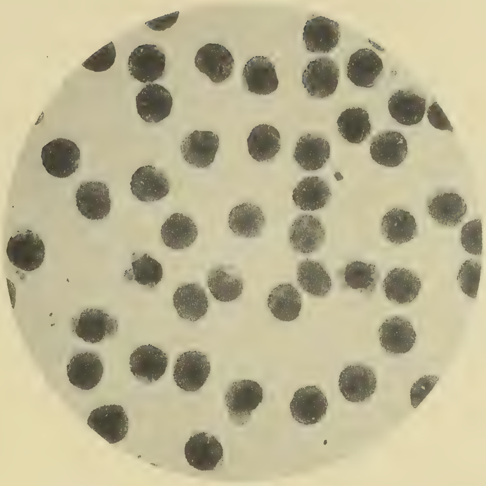

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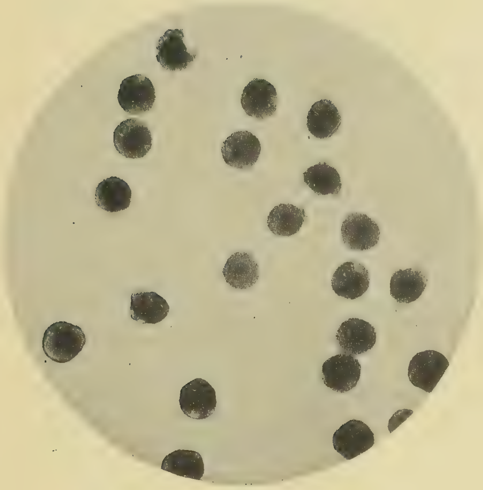

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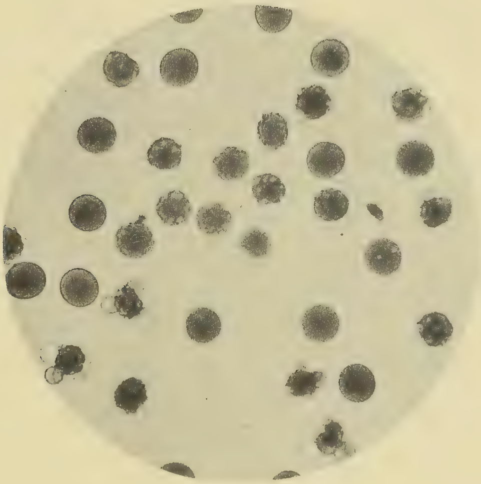

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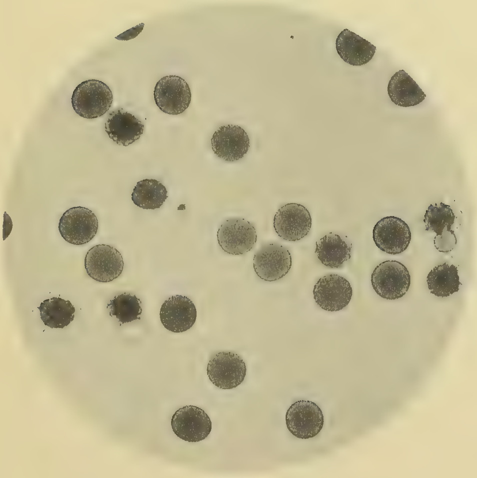

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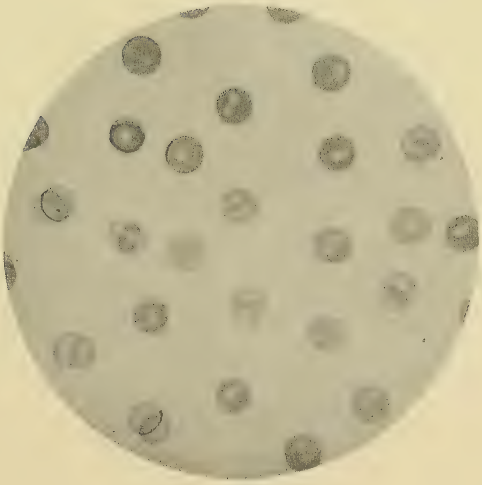

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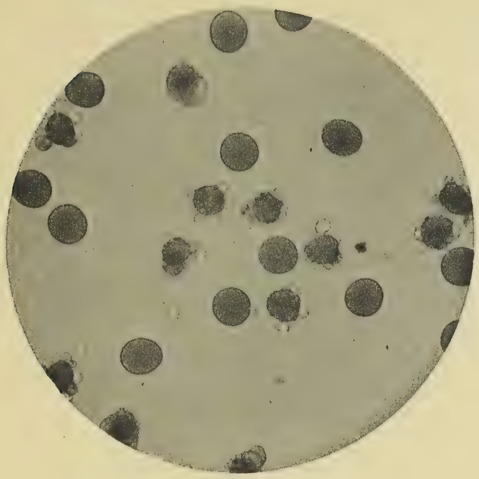
PLATE 2

Neutral Red 1:150,000 — *Continued*

FIG. 1. Grübler lot I. 10-minute irradiation. 51 minutes after insemination.
FIG. 2. Grübler lot I. 15-minute irradiation. 53 minutes after insemination.
FIG. 3. Control. 54 minutes after insemination.
FIG. 4. Grübler lot I. Darkness. 56 minutes after insemination.
FIG. 5. Grübler lot I. 10-minute irradiation. 58 minutes after insemination.
FIG. 6. Grübler lot I. 15-minute irradiation. 61 minutes after insemination.



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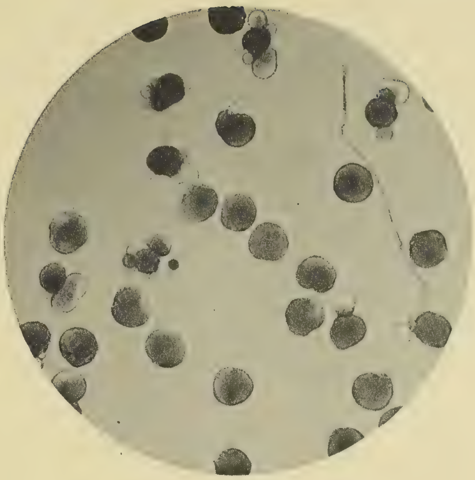

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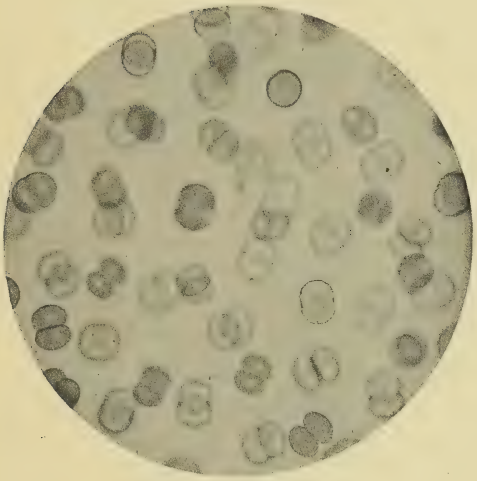

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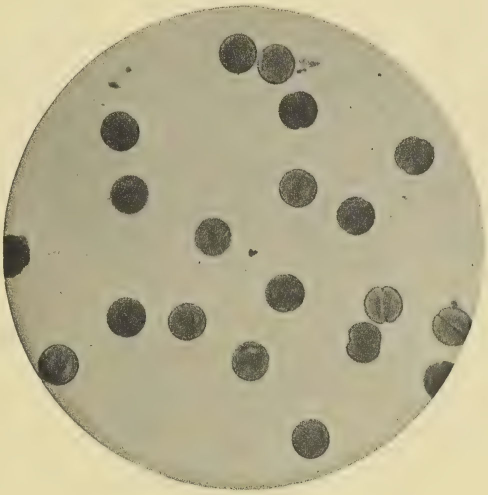

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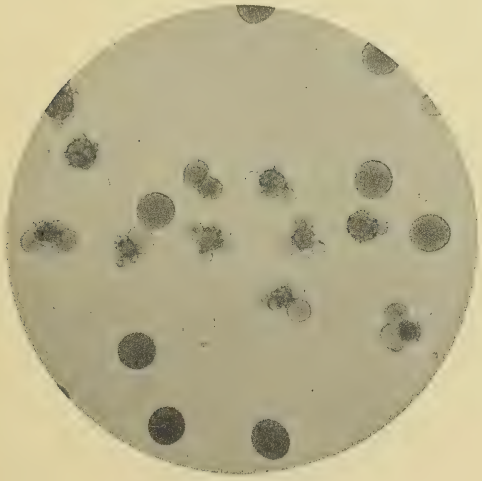

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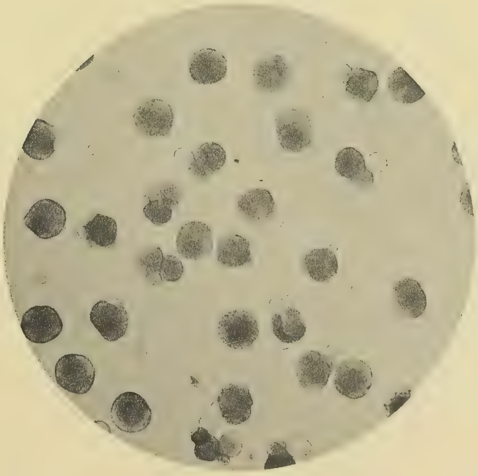

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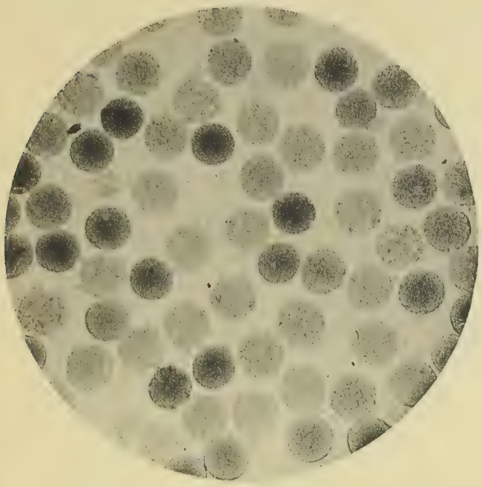
PLATE 3

Magdala Red echt (see text page 78)

FIG. 1. 1:20,000. 15-minute irradiation. 54 minutes after insemination.
FIG. 2. Control. 61 minutes after insemination.

Brilliant Green 1:800,000 (see text page 104)

FIG. 3. 20-minute irradiation. 11 minutes after insemination.
FIG. 4. 20-minute irradiation. 19 minutes after insemination.
FIG. 5. 20-minute irradiation. 22 minutes after insemination.
FIG. 6. 20-minute irradiation. 24 minutes after insemination.



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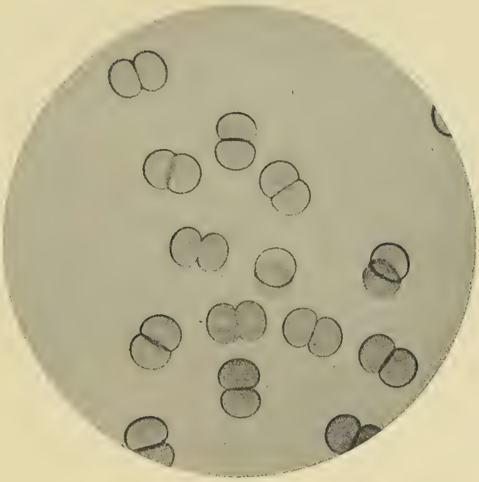

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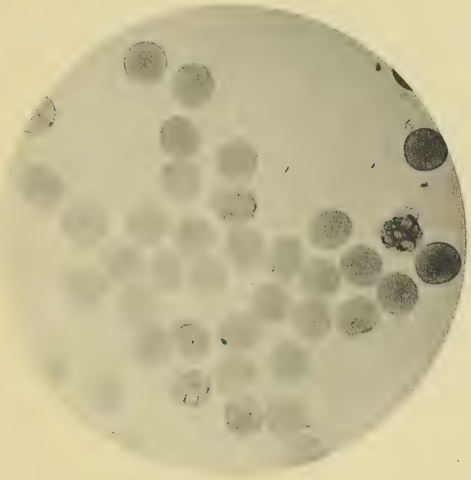

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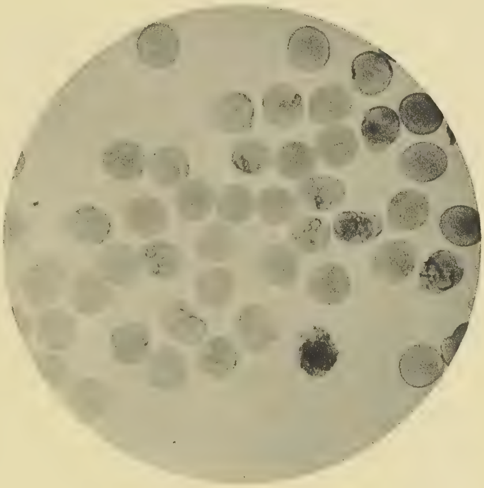

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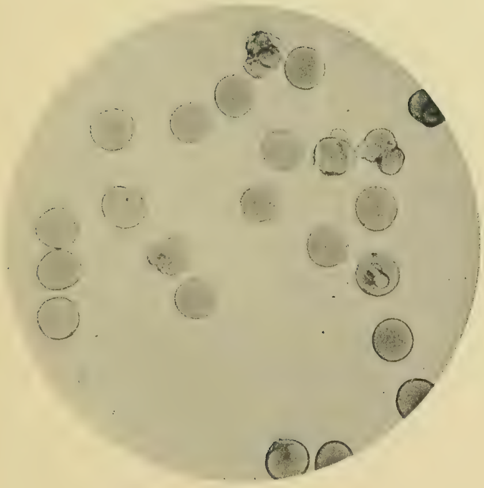

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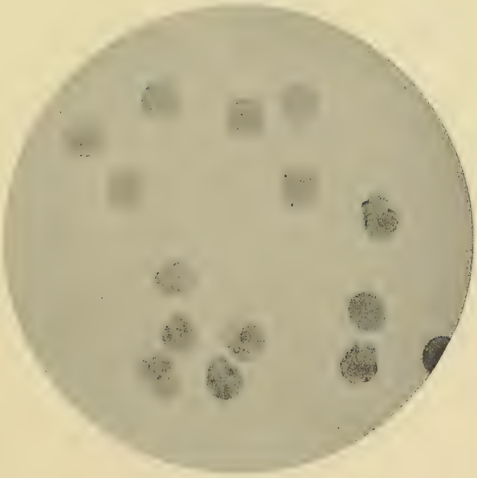

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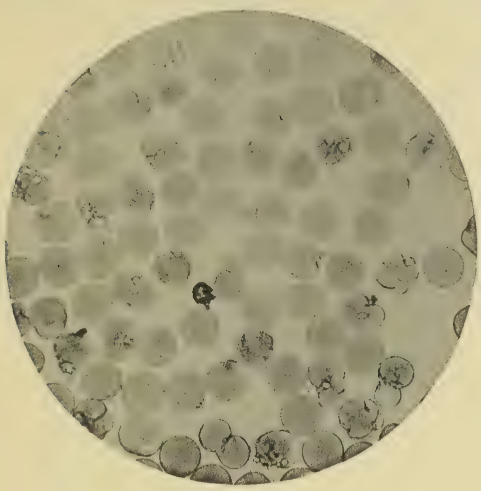
PLATE 4

Brilliant Green 1:800,000 — *Continued*

FIG. 1. 20-minute irradiation. 38 minutes after insemination.
FIG. 2. 20-minute irradiation. 44 minutes after insemination.
FIG. 3. 20-minute irradiation. 52 minutes after insemination.
FIG. 4. 20-minute irradiation. 82 minutes after insemination.
FIG. 5. 20-minute irradiation. 97 minutes after insemination.

Eosin Y (see text pages 128–130)

FIG. 6. 1:50,000. Darkness. 53 minutes after insemination.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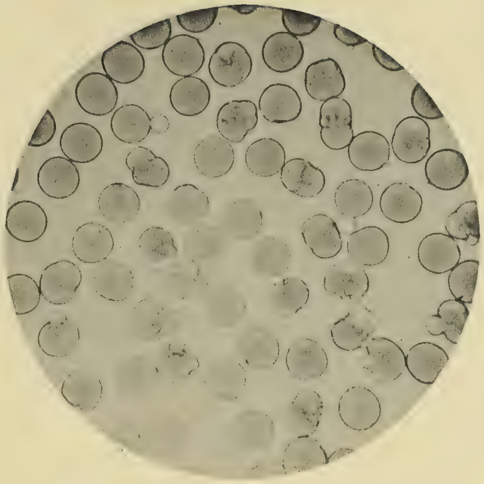
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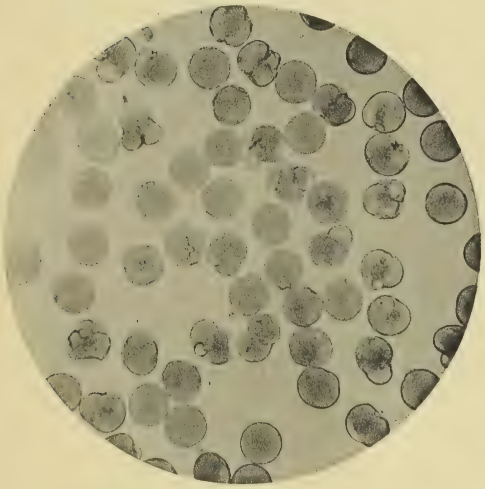
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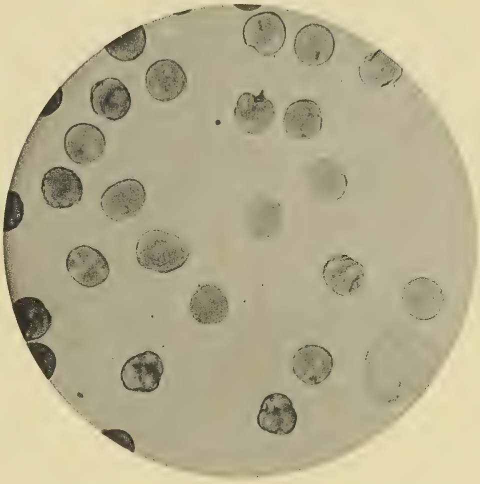
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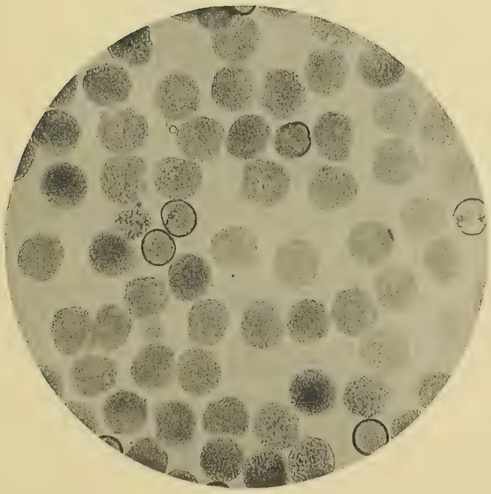
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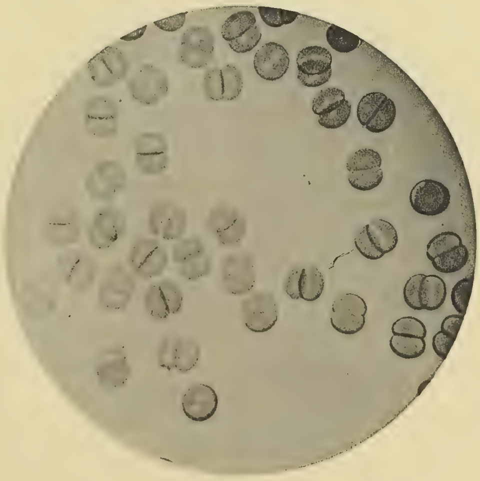
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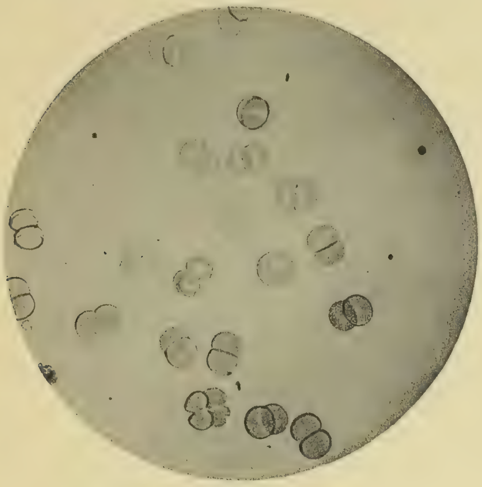
PLATE 5

Eosin Y — *Continued*

FIG. 1. Control. 60 minutes after insemination.
FIG. 2. 1:50,000. 10-minute irradiation. 65 minutes after insemination.
FIG. 3. 1:100,000. 20-minute irradiation. 18 minutes after insemination.
FIG. 4. 1:50,000. 20-minute irradiation. 20 minutes after insemination.
FIG. 5. 1:50,000. 20-minute irradiation. 22 minutes after insemination.
FIG. 6. 1:150,000. 20-minute irradiation. 47 minutes after insemination.



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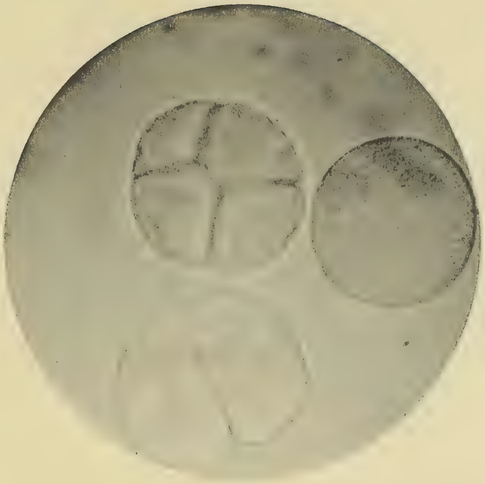

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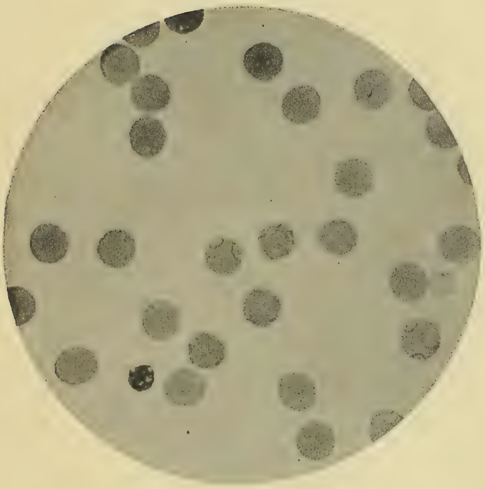

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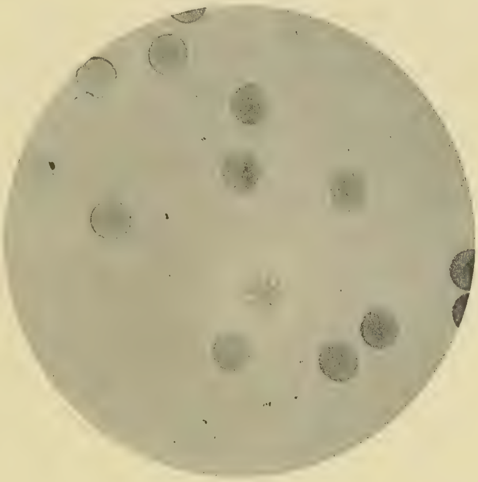

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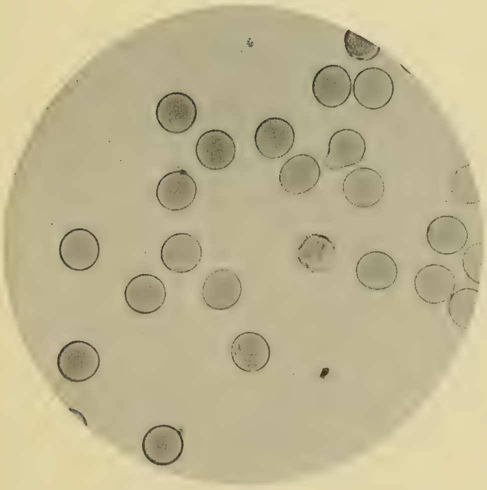

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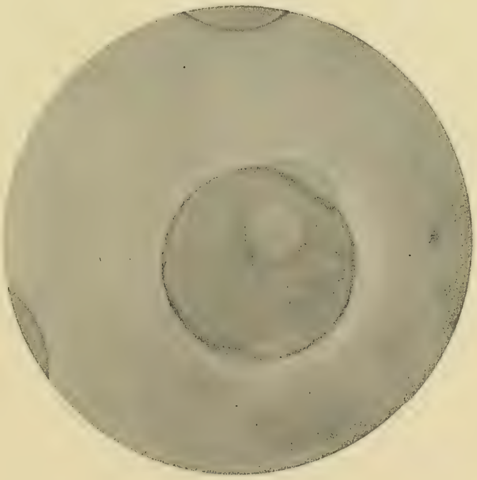
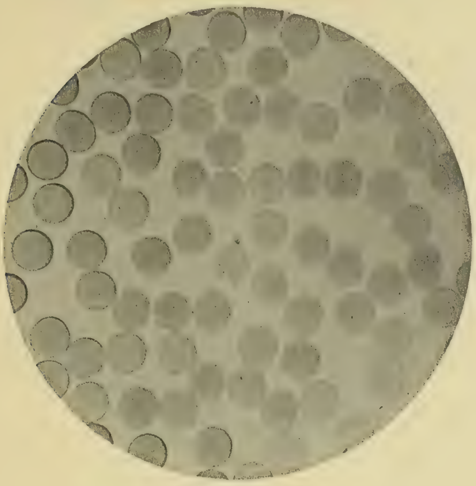


6

Plate 6

Eosin Y — *Continued*

Fig. 1. 1:150,000. 20-minute irradiation. 17 minutes after insemination.
Fig. 2. 1:150,000. Darkness. 22 minutes after insemination.
Fig. 3. 1:150,000. 20-minute irradiation. 27 minutes after insemination.
Fig. 4. 1:150,000. 20-minute irradiation. 32 minutes after insemination.
Fig. 5. 1:150,000. 20-minute irradiation. 57 minutes after insemination.
Fig. 6. 1:150,000. 20-minute irradiation. 61 minutes after insemination.



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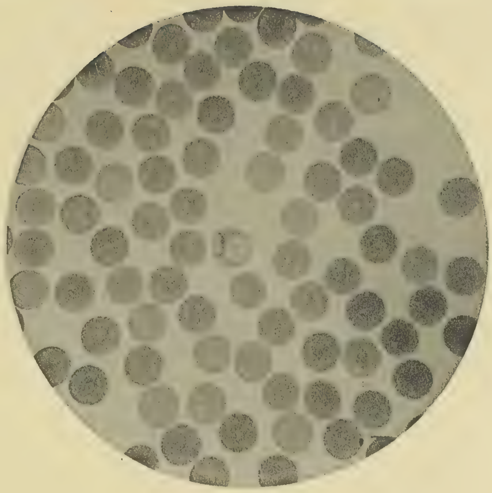

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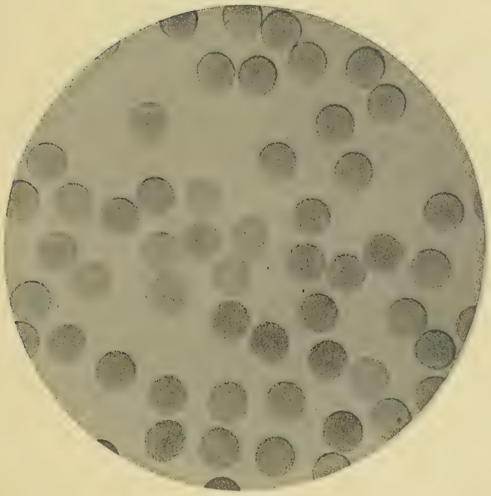

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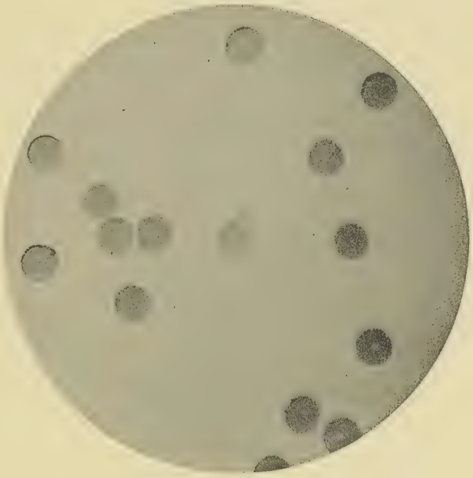

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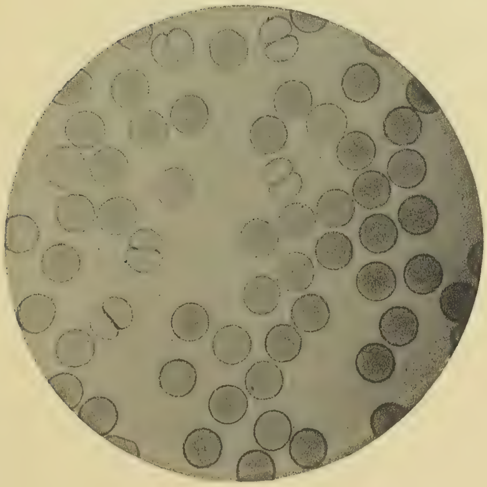

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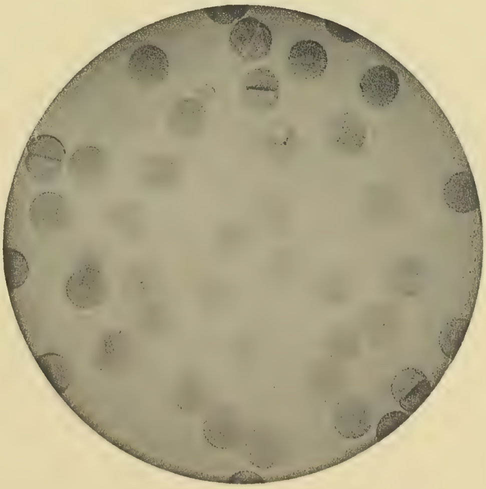
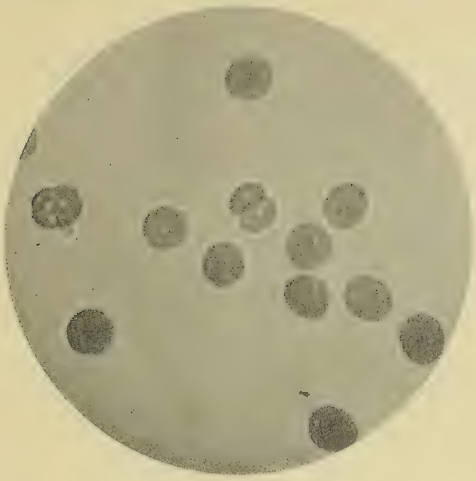


6

PLATE 7

Phloxine 1:40,000 (see text page 133)

Fig. 1. Darkness. 64 minutes after insemination.
Fig. 2. 15-minute irradiation. 64 minutes after insemination.
Fig. 3. 15-minute irradiation. 65 minutes after insemination.
Fig. 4. Darkness. 1 hour and 57 minutes after insemination.
Fig. 5. 10-minute irradiation. 1 hour and 53 minutes after insemination.
Fig. 6. 15-minute irradiation. 1 hour and 57 minutes after insemination.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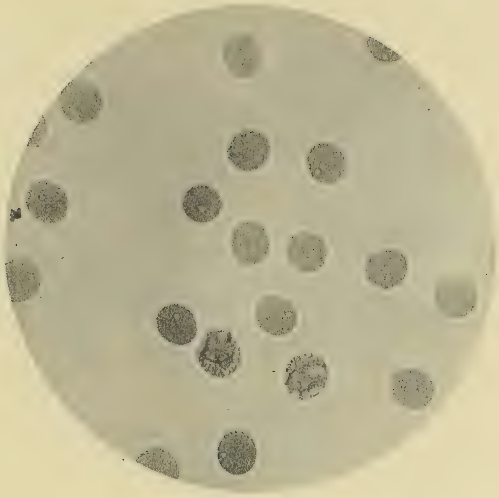
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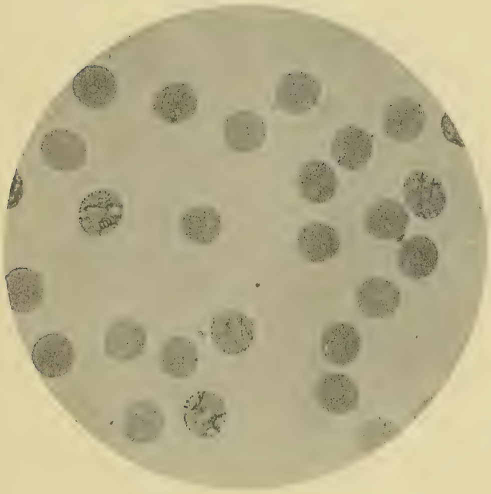
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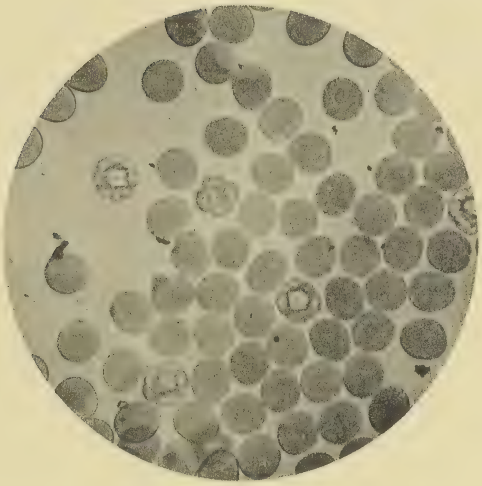
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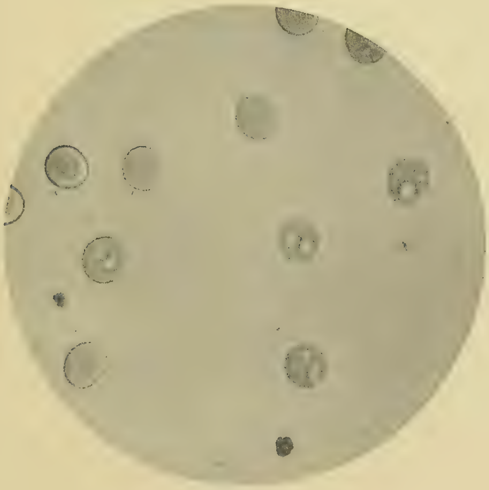
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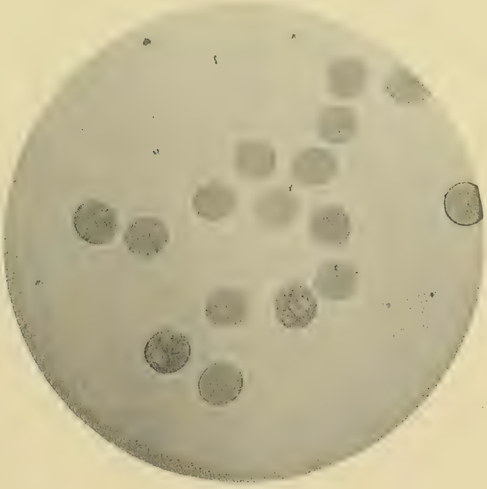
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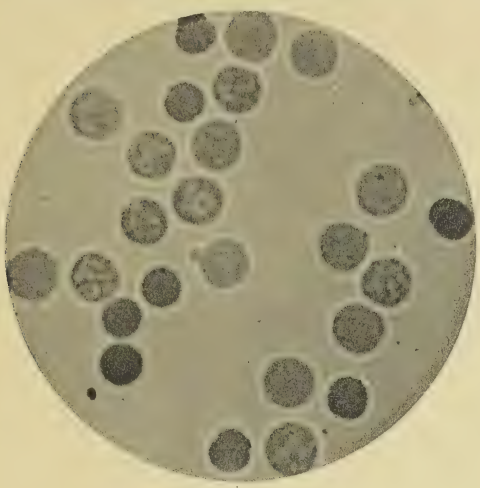
PLATE 8

Phloxine Red 1:40,000 (see text page 135)

FIG. 1. 10-minute irradiation. 61 minutes after insemination.
FIG. 2. 15-minute irradiation. 57 minutes after insemination.
FIG. 3. 15-minute irradiation. 58 minutes after insemination.
FIG. 4. 10-minute irradiation. 2 hours and 7 minutes after insemination.
FIG. 5. 15-minute irradiation. 2 hours and 4 minutes after insemination.
FIG. 6. Control. 2 hours and 10 minutes after insemination.



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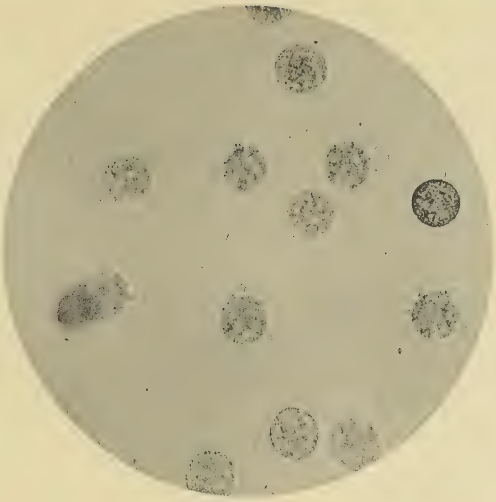

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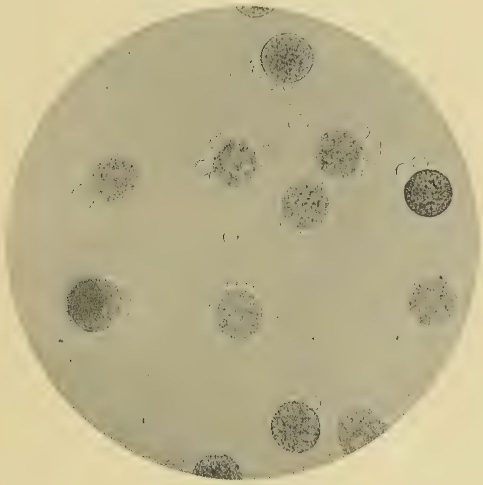

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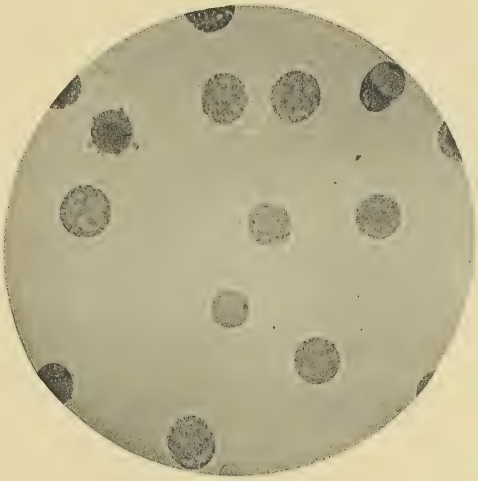

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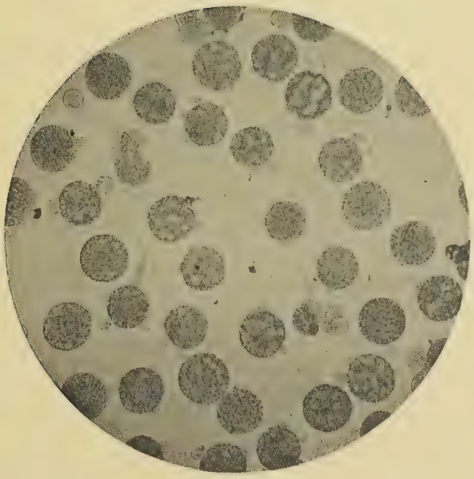

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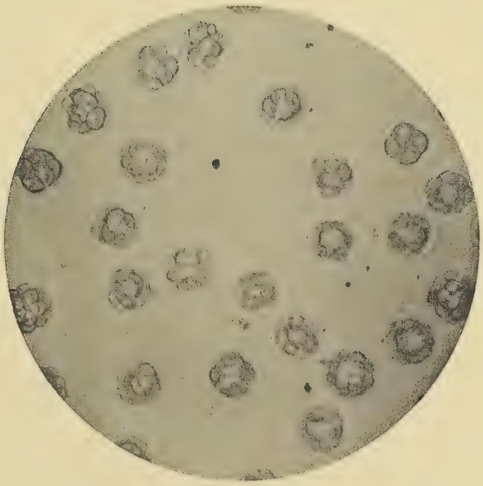

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